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(54) **Cell-permeable peptide inhibitors of the JNK signal transduction pathway**

Zelldurchlässige Peptidhemmer des JNK-Signaltransduktionsweges

Inhibiteurs peptidiques à perméabilité cellulaire du processus de transduction du signal de JNK

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(56) References cited:
EP-A- 0 897 002 WO-A-94/04686
WO-A-98/44106 WO-A-98/49188

- **DICKENS M ET AL.:** "A cytoplasmic inhibitor of the JNK signal transduction pathway" **SCIENCE**, vol. 277, no. 5326, 1 August 1997 (1997-08-01), pages 693-696, XP002172257
- **YASUDA J ET AL.:** "The JIP group of mitogen-activated protein kinase scaffold proteins" **MOLECULAR AND CELLULAR BIOLOGY**, vol. 19, no. 10, October 1999 (1999-10), pages 7245-7254, XP002172259
- **BONNY C ET AL.:** "IB1, a JIP-1-related nuclear protein present in insulin-secreting cells" **JOURNAL OF BIOLOGICAL CHEMISTRY**, vol. 273, no. 4, 23 January 1998 (1998-01-23), pages 1843-1846, XP002172258
- **VIVÈS E ET AL.:** "A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus." **JOURNAL OF BIOLOGICAL CHEMISTRY**, vol. 272, no. 25, 20 June 1997 (1997-06-20), pages 16010-16017, XP002172260
- **BONNY C ET AL.:** "Cell-permeable peptide inhibitors of JNK: novel blockers of beta-cell death." **DIABETES**, vol. 50, no. 1, January 2001 (2001-01), pages 77-82, XP002172261

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Description**FIELD OF THE INVENTION**

5 [0001] This invention relates generally to protein kinase inhibitors and more specifically to inhibitors of the protein kinase c-Jun amino terminal kinase.

BACKGROUND OF THE INVENTION

10 [0002] The c-Jun amino terminal kinase (JNK) is a member of the stress-activated group of mitogen-activated protein (MAP) kinases. These kinases have been implicated in the control of cell growth and differentiation, and, more generally, in the response of cells to environmental stimuli. The JNK signal transduction pathway is activated in response to environmental stress and by the engagement of several classes of cell surface receptors. These receptors can include cytokine receptors, serpentine receptors, and receptor tyrosine kinases. In mammalian cells, JNK has been implicated
15 in such biological processes as oncogenic transformation and in mediating adaptive responses to environmental stress. JNK has also been associated with modulating immune responses, including maturation and differentiation of immune cells, as well effecting programmed cell death in cells identified for destruction by the immune system.

SUMMARY OF THE INVENTION

20 [0003] The present invention is based in part on the discovery of peptides that are effective inhibitors of JNK proteins. The peptides, referred to herein as JNK peptide inhibitors, decrease the downstream cell-proliferative effects of c-Jun amino terminal kinase (JNK).

[0004] Accordingly, the invention includes novel chimeric peptides which include a JNK peptide inhibitor linked a
25 trafficking peptide that can be used to direct a peptide on which it is present to a desired cellular location. The trafficking sequence

can be used to direct transport of the peptide across the plasma membrane. Alternatively, or in addition, the trafficking peptide can be used to direct the peptide to desired intracellular location, such as the nucleus.

[0005] The JNK inhibitor peptides can be present as polymers of L-amino acids. Alternatively, the peptides can be
30 present as polymers of D-amino acids.

[0006] Also included in the invention are pharmaceutical compositions that include the JNK-binding peptides, as well as antibodies that specifically recognize the JNK-binding peptides.

[0007] In another aspect, the invention includes the use of the inventive peptides for the manufacture of a medicament
35 for treating a pathophysiology associated with activation of JNK in a cell or cells. For example, the target cells may be, e.g., cultured animal cells, human cells or micro-organisms. Delivery can be carried out *in vivo* by administering the chimeric peptide to an individual in whom it is to be used for diagnostic, preventative or therapeutic purposes. The target cells may be *in vivo* cells, i.e., cells composing the organs or tissues of living animals or humans, or microorganisms found in living animals or humans.

[0008] Among the advantages provided by the invention is that the JNK inhibitor peptides are small, and can be
40 produced readily in bulk quantities and in high purity. The inhibitor peptides are also resistant to intracellular degradation, and are weakly immunogenic. Accordingly, the peptides are well suited for *in vitro* and *in vivo* applications in which inhibition of JNK-expression is desired.

[0009] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar
45 or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

50 [0010] Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS**[0011]**

55 FIGS. 1 A-C are diagrams showing alignments of conserved JBD domain regions in the indicated transcription factors.

FIG. 2 is a diagram showing alignments of generic TAT-IB fusion peptides.

FIG. 3 is a histogram depicting inhibition of β -cell death by the minimal 23 amino acid long JBD domain of IB1 compared to the full 280 amino acid JBD domain.

FIG. 4 is an illustration demonstrating the effects of TAT, TAT-IB1 and TAT-IB2 peptides on phosphorylation of recombinant JNKs. Panel A shows inhibition of c-Jun, ATF2 and Elk1 phosphorylation by recombinant JNKs *in vitro*. Panel B shows dose response experiments similar to Panel A.

FIG. 5 is a histogram depicting L-TAT-IB inhibition of phosphorylation by recombinant JNKs. Panel A shows L-TAT-IB inhibition of c-Jun, ATF2 and Elk1 phosphorylation by recombinant JNKs *in vitro* in the presence of MKK4. Panel B shows similar dose response experiments with MKK7.

FIG. 6 is an illustration demonstrating the inhibition of c-Jun phosphorylation by activated JNKs.

FIG. 7 is a histogram depicting short term inhibition of IL-1 β induced pancreatic β -cell death by the L-TAT-IB peptides.

FIG. 8 is a histogram depicting short term inhibition of IL-1 β induced pancreatic β -well death by the D-TAT-IB peptides.

FIG. 9 is a histogram depicting long term inhibition of IL-1 β induced pancreatic β -cell death by L-TAT-IB 1 and D-TAT-IB1 peptides.

FIG. 10 is a histogram depicting inhibition of irradiation induced human colon cancer WiDr cell death by L-TAT-IB1 and D-TAT-IB1 peptides.

FIG. 11 is an illustration of the modulation of JNK kinase activity by L-TAT, TAT-IB1 and D-TAT-IB1 peptides.

FIG. 12 are graphs depicting the protective effects of the TAT-IB peptides in mice. Panel A shows the effect of irradiation on weight. Panel B shows the effect of irradiation on oedema and erythema status.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The present invention is based in part on the discovery of cell permeable peptides that inhibit the activated c-Jun amino terminal kinase (JNK) signaling pathway. These peptides are referred to herein as JNK inhibitor peptides. Additionally, the discovery provides methods and pharmaceutical compositions for treating pathophysiologies associated with JNK signaling.

[0013] JNK inhibitor peptides were identified by inspecting sequence alignments between kJNK Binding Domains in various insulin binding (IB) proteins. The results of this alignment are shown in FIGS. 1A-1C. FIG. 1A depicts the region of highest homology between the JBDs of IB1, IB2, c-Jun and ATF2. Panel B depicts the amino acid sequence alignment of the JBDs of IB1 and IB2. Fully conserved residues are indicated by asterisks, while residues changed to Ala in the GFP-JBD_{23Mut} vector are indicated by open circles. FIG. 1C shows the amino acid sequences of chimeric proteins that include a JNK inhibitor peptide domain and a trafficking domain. In the example shown, the trafficking domain is derived from the human immunodeficiency virus (HIV) TAT polypeptide, and the JNK inhibitor peptide is derived from an IB1 polypeptide. Human, mouse, and rat sequences are identical in Panels B and C.

[0014] Sequence comparison between the JNK binding domains of IB1 [SEQ ID NO: 17], IB2 [SEQ ID NO: 18], c-Jun [SEQ ID NO: 19] and ATF2 [SEQ ID NO: 20] revealed a partially conserved 8 amino acid sequence (FIG. 1A). A comparison of the JBDs of IB1 and IB2 further revealed two blocks of seven and three amino acids that are highly conserved between the two sequences. These two blocks are contained within a peptide sequence of 23 amino acids in IB1 [SEQ ID NO: 1] and 21 amino acids in IB2 [SEQ ID NO: 2].

[0015] The JNK inhibitor peptides of the invention can be used in any situation in which inhibition of JNK activity is desired. This can include *in vitro* applications, *ex vivo*, and *in vivo* applications. As JNKs and all its isoforms participate in the development and establishment of pathological states or in pathways, the JNK peptides can be used to prevent or inhibit the occurrence of such pathological states. This includes prevention and treatment of diseases and prevention and treatment of conditions secondary to therapeutic actions. For example, the peptides of the present invention can be used to treat or prevent, *e.g.*, diabetes, ionizing radiation, immune responses (including autoimmune diseases), ischemia/reperfusion injuries, heart and cardiovascular hypertrophies, and some cancers (*e.g.*, *Bcr-Abl* transformation).

[0016] The peptides can also be used to inhibit expression of genes whose expression increases in the presence of an active JNK polypeptide. These genes and gene products includes, *e.g.*, proinflammatory cytokines. Such cytokines are found in all forms of inflammatory, auto-inflammatory, immune and autoimmune diseases, degenerative diseases, myopathies, cardiomyopathies, and graft rejection.

[0017] The JNK inhibitor peptides described herein can also be used to treat or prevent effects associated with cellular shear stress, such as in pathological states induced by arterial hypertension, including cardiac hypertrophy and arteriosclerotic lesions, and at bifurcations of blood vessels, and the like; ionizing radiation, as used in radiotherapy and UV lights; free radicals; DNA damaging agents, including chemotherapeutic drugs; oncogenic transformation; ischemia and reperfusion; hypoxia; and hypo- and hyperthermia

[0018] The polynucleotides provided by the present invention can be used to express recombinant peptides for therapeutic use; 15. Other uses for the nucleic acids include, e.g., molecular weight markers in gel electrophoresis-based analysis of nucleic acids.

[0019] The JNK inhibitor peptides disclosed herein are presented in Table 1. The table presents the name of the JNK inhibitor peptide, as well as its sequence identifier number, length, and amino acid sequence.

TABLE 1

PEPTIDE NAME	SEQ ID	A A	Sequence
L-IB1	1	23	DTYRPKRPTT LNLFPQVPRS QDT
L-IB2	2	21	EEPHKHRPTT LRLTTLGAQD S
D-IB1	3	23	TDQSRPVQPF LNLTPRKPR YTD
D-IB2	4	21	SDQAGLTTLR LTPRHKHPE E
L-IB (generic)	5	19	XRPTTLXLXX XXXXXQDS/TX
D-IB (generic)	6	19	XS/TDQXXXXXX XLXLTPRXX
L-TAT	7	10	GRKKRRQRRR
D-TAT	8	10	RRRQRKKRG
L-generic-TAT	9	17	XXXXRKKRRQ RRRXXXX
D-generic-TAT	10	17	XXXXRRRQRR KKRXXXX
L-TAT-IB1	11	35	GRKKRRQRRR PPDTYRPKRP TTLNLFPQVP RSQDT
L-TAT-IB2	12	33	GRKKRRQRRR PPEPHKHRP TTLRLTTLGA QDS
L-TAT-IB (generic)	13	42	XXXXXXXXRK RRQRRRXXX XXXXRPTTLX LXXXXXXXXQD S/TX
D-TAT-IB1	14	35	TDQSRPVQPF LNLTPRKPR YDPPRRRQR RKKRG
D-TAT-IB2	15	33	SDQAGLTTLR LTPRHKHPE EPPRRRQRRK KRG
D-TAT-IB (generic)	16	42	XT/SDQXXXXXX XLXLTPRXX XXXXXRRRQ RKKRXXXXXX XX
IB1-long	17	29	PGTGCGDTYR PKRPTTLNLF PQVPRSQDT
IB2-long	18	27	IPSPSVEEPH KHRPTTLRLT TLGAQDS
c-Jun	19	29	GAYGYSNPKI LKQSMTLNLA DPVGNLKPH
A1F2	20	29	TNEDHLAVHK HKHEMTLKFG PARNDSVIV

JNK INHIBITOR PEPTIDES

[0020] In one aspect, the invention provides a JNK inhibitor peptide. The JNK-inhibitor peptide may be less than 25 amino acids in length. In various embodiments, the JNK-binding inhibitor peptide includes the amino acid sequence of one of SEQ ID NOs: 1, 3, 5 or 6. In one embodiment, the JNK inhibitor peptide peptides bind JNK. The peptide may inhibit the activation of at least one JNK activated transcription factor, e.g. c-Jun, ATF2 or Elk1.

[0021] Examples of JNK inhibitor peptides include a peptide which includes (in whole or in part) the sequence NH_2 -DTYRPKRPTTLNLFPQVPRSQDT-cooH [SEQ ID NO:1].

[0022] The JNK inhibitor peptides can be polymers of L-amino acids, D-amino acids, or a combination of both. For example, in various embodiments, the peptides are D retro-inverso peptides. The term "retro-inverso isomer" refers to an isomer of a linear peptide in which the direction of the sequence is reversed and the chirality of each amino acid residue is inverted. See, e.g., Jameson et al., Nature, 368, 744-746 (1994); Brady et al., Nature, 368, 692-693 (1994). The net result of combining D-enantiomers and reverse synthesis is that the positions of carbonyl and amino groups in each amide bond are exchanged, while the position of the side-chain groups at each alpha carbon is preserved. Unless

specifically stated otherwise, it is presumed that any given L-amino acid sequence of the invention may be made into an D retro-inverso peptide by synthesizing a reverse of the sequence for the corresponding native L-amino acid sequence.

[0023] AD retro-inverso peptide may have the sequence
 $\text{NH}_2\text{-TDQSRPVQPFLNLTTPRICPRYTD-cooH}$ [SEQ ID NO:3].

It has been unexpectedly found that D-retro-inverso TAT-IB peptides have a variety of useful properties. For example, D-TAT and D-TAT-IB peptides enter cells as efficiently as L- TAT and L-TAT-IB peptides, and D-TAT and D-TAT-IB peptides are more stable than the corresponding L-peptides. Further, while D-TAT-IB1 are ~10-20 fold less efficient in inhibiting JNK than L-TAT-IB, they are ~50 fold more stable *in vivo*. Finally, as it is discussed further below, D-TAT-IB peptides protect interleukin-1 treated and ionizing irradiated cells from apoptosis.

[0024] AJNK inhibitor peptide according to the invention includes the amino acid sequence $\text{NH}_2\text{-X}_n\text{-RPTTLX-LXXXXXXQD/T-X}_n\text{-cooH}$ [SEQ ID NO: 5, and residues 17-42 of L-TAT-IB, SEQ ID NO: 13, as shown in FIG.2]. As used herein, X_n may be zero residues in length, or maybe a contiguous stretch of peptide residues derived from SEQ ID NO: 1, preferably a stretch of between 1 and 7 amino acids in length, or may be 10, 20 or more amino acids in length. The single residue represented by S/T may be either Ser or Thr in the generic sequence. The generic IB peptide may be a D retro-inverso peptide having the sequence $\text{NH}_2\text{-X}_n\text{-S/TDQXXXXXXXXLXLTPR-X}_n\text{-cooH}$ [SEQ ID NO: 6, and residues 17-42 of L-TAT-IB, SEQ ID NO:16, as shown in FIG.2].

[0025] JNK-inhibitor peptides may be obtained or produced by methods well-known in the art, e.g. chemical synthesis, genetic engineering methods as discussed below. For example, a peptide corresponding to a portion of a JNK inhibitor peptide including a desired region or domain, or that mediates the desired activity *in vitro*, may be synthesized by use of a peptide synthesizer.

[0026] A candidate JNK inhibitor peptide may also be analyzed by hydrophilicity analysis (see, e.g., Hopp and Woods, 1981. Proc Natl Acad Sci USA 78: 3824-3828) that can be utilized to identify the hydrophobic and hydrophilic regions of the peptides, thus aiding in the design of substrates for experimental manipulation, such as in binding experiments, antibody synthesis. Secondary structural analysis may also be performed to identify regions of AJNK inhibitor peptide that assume specific structural motifs. See e.g., Chou and Fasman, 1974. Biochem 13: 222-223. Manipulation, translation, secondary structure prediction, hydrophilicity and hydrophobicity profiles, open reading frame prediction and plotting, and determination of sequence homologies can be accomplished using computer software programs available in the art. Other methods of structural analysis including, e.g., X-ray crystallography (see, e.g., Engstrom, 1974. Biochem Exp Biol 11:7-13); mass spectroscopy and gas chromatography (see, e.g., METHODS IN PROTEIN SCIENCE, 1997. J. Wiley and Sons, New York, NY) and computer modeling (see, e.g., Fletterick and Zoller, eds., 1986. Computer Graphics and Molecular Modeling, In: CURRENT COMMUNICATIONS IN MOLECULAR BIOLOGY, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) may also be employed.

[0027] The present invention additionally relates to nucleic acids that encode JNK-binding peptides having L-form amino acids, e.g., those L-peptides indicated in Table 1, as well as the complements of these sequences. Suitable sources of nucleic acids encoding JNK inhibitor peptides include the human IB 1 nucleic acid (and the encoded protein sequences) available as GenBank Accession Nos. AF074091 and AAD20443, respectively. Other sources include rat IB1 nucleic acid and protein sequences are shown in GenBank Accession No. AF108959 and AAD22543, respectively, and are incorporated herein by reference in their entirety. Human m2 nucleic acid and protein sequences are shown in GenBank Accession No AF218778 and is also incorporated herein by reference in their entirety.

[0028] Nucleic acids encoding the JNK inhibitor peptides may be obtained by any method known in the art (e.g., by PCR amplification using synthetic primers hybridizable to the 3'-and 5'-termini of the sequence and/or by cloning from a cDNA or genomic library using an oligonucleotide sequence specific for the given gene sequence).

[0029] For recombinant expression of one or more JNK inhibitor peptides, the nucleic acid containing all or a portion of the nucleotide sequence encoding the peptide may be inserted into an appropriate expression vector (i.e., a vector that contains the necessary elements for the transcription and translation of the inserted peptide coding sequence). The regulatory elements are heterologous (i.e., not the native gene promoter). Alternately, the necessary transcriptional and translational signals may also be supplied by the native promoter for the genes and/or their flanking regions.

[0030] A variety of host-vector systems may be utilized to express the peptide coding sequence(s). These include, but are not limited to: (i) mammalian cell systems that are infected with vaccinia virus, adenovirus, and the like; (ii) insect cell systems infected with baculovirus and the like; (iii) yeast containing yeast vectors or (iv) bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

[0031] Promoter/enhancer sequences within expression vectors may utilize plant, animal, insect, or fungus regulatory sequences, as provided in the invention. For example, promoter/enhancer elements can be used from yeast and other fungi (e.g., the GAL4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter). Alternatively, or in addition, they may include animal transcriptional control regions, e.g., (i) the

insulin gene control region active within pancreatic β -cells (see, e.g., Hanahan, et al., 1985. Nature 315: 115-122); (ii) the immunoglobulin gene control region active within lymphoid cells (see, e.g., Grosschedl, et al., 1984. Cell 38: 647-658); (iii) the albumin gene control region active within liver (see, e.g., Pinckert, et al., 1987. Genes and Dev 1: 268-276; (iv) the myelin basic protein gene control region active within brain oligodendrocyte cells (see, e.g., Readhead, et al., 1987. Cell 48: 703-712); and (v) the gonadotropin-releasing hormone gene control region active within the hypothalamus (see, e.g., Mason, et al., 1986. Science 234: 1372-1378), and the like.

[0032] Expression vectors or their derivatives include, e.g. human or animal viruses (e.g., vaccinia virus or adenovirus); insect viruses (e.g., baculovirus); yeast vectors; bacteriophage vectors (e.g., lambda phage); plasmid vectors and cosmid vectors.

[0033] A host cell strain may be selected that modulates the expression of inserted sequences of interest, or modifies or processes expressed peptides encoded by the sequences in the specific manner desired. In addition, expression from certain promoters may be enhanced in the presence of certain inducers in a selected host strain; thus facilitating control of the expression of a genetically-engineered peptides. Moreover, different host cells possess characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation, and the like) of expressed peptides. Appropriate cell lines or host systems may thus be chosen to ensure the desired modification and processing of the foreign peptide is achieved. For example, peptide expression within a bacterial system can be used to produce an unglycosylated core peptide; whereas expression within mammalian cells ensures "native" glycosylation of a heterologous peptide.

[0034] Also included in the invention are derivatives, fragments, homologs, analogs and variants of JNK inhibitor peptides and nucleic acids encoding these peptides. For nucleic acids, derivatives, fragments, and analogs provided herein are defined as sequences of at least 6 (contiguous) nucleic acids, and which have a length sufficient to allow for specific hybridization. For amino acids, derivatives, fragments, and analogs provided herein are defined as sequences of at least 4 (contiguous) amino acids, a length sufficient to allow for specific recognition of an epitope.

[0035] The length of the fragments are less than the length of the corresponding full-length nucleic acid or polypeptide from which the JNK inhibitor peptide, or nucleic acid encoding same, is derived. Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid. Derivatives or analogs of the JNK inhibitor peptides include, e.g., molecules including regions that are substantially homologous to the peptides, by at least 80%, or 95%, 98%, or even 99%, identity over an amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art. For example sequence identity can be measured using sequence analysis software (Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705), with the default parameters therein.

[0036] In the case of polypeptide sequences, which are less than 100% identical to a reference sequence, the non-identical positions are preferably, but not necessarily, conservative substitutions for the reference sequence. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Thus, included in the invention are peptides having mutated sequences such that they remain homologous, e.g. in sequence, in function, and in antigenic character or other function, with protein having the corresponding parent sequence. Such mutations can, for example, be mutations involving conservative amino acid changes, e.g., changes between amino acids of broadly similar molecular properties. For example, interchanges within the aliphatic group alanine, valine, leucine and isoleucine can be considered as conservative. Sometimes substitution of glycine for one of these can also be considered conservative. Other conservative interchanges include those within the aliphatic group aspartate and glutamate; within the amide group asparagine and glutamine; within the hydroxyl group serine and threonine; within the aromatic group phenylalanine, tyrosine and tryptophan; within the basic group lysine, arginine and histidine; and within the sulfur-containing group methionine and cysteine. Sometimes substitution within the group methionine and leucine can also be considered conservative. Preferred conservative substitution groups are aspartate-glutamate; asparagine-glutamine; valine-leucine-isoleucine; alanine-valine; phenylalanine-tyrosine; and lysine-arginine.

[0037] Where a particular polypeptide is said to have a specific percent identity to a reference polypeptide of a defined length, the percent identity is relative to the reference peptide. Thus, a peptide that is 50% identical to a reference polypeptide that is 100 amino acids long can be a 50 amino acid polypeptide that is completely identical to a 50 amino acid long portion of the reference polypeptide. It might also be a 100 amino acid long polypeptide, which is 50% identical to the reference polypeptide over its entire length. Of course, other polypeptides will meet the same criteria.

[0038] The invention also encompasses allelic variants of the disclosed polynucleotides or peptides; that is, naturally-occurring alternative forms of the isolated polynucleotide that also encode peptides that are identical, homologous or related to that encoded by the polynucleotides. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

[0039] Species homologs of the disclosed polynucleotides and peptides are also provided by the present invention.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and in many regions, identical to the polynucleotide or polypeptide of the present invention. The variants may contain alterations in the coding regions, non-coding regions, or both.

[0040] In some embodiments, altered sequences include insertions such that the overall amino acid sequence is lengthened while the protein retains trafficking properties. Additionally, altered sequences may include random or designed internal deletions that shorten the overall amino acid sequence while the protein retains transport properties.

[0041] The altered sequences can additionally or alternatively be encoded by polynucleotides that hybridize under stringent conditions with the appropriate strand of the naturally-occurring polynucleotide encoding a polypeptide or peptide from which the JNK inhibitor peptide is derived. The variant peptide can be tested for JNK-binding and modulation of JNK-mediated activity using the herein described assays. 'Stringent conditions' are sequence dependent and will be different in different circumstances. Generally, stringent conditions can be selected to be about 5°C lower than the thermal melting point (T_M) for the specific sequence at a defined ionic strength and pH. The T_M is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60°C. As other factors may affect the stringency of hybridization (including, among others, base composition and size of the complementary strands), the presence of organic solvents and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one.

[0042] High stringency can include, e.g., Step 1: Filters containing DNA are pretreated for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Step 2: Filters are hybridized for 48 hours at 65°C in the above prehybridization mixture to which is added 100 mg/ml denatured salmon sperm DNA and 5-20 x 10⁶ cpm of ³²P-labeled probe. Step 3: Filters are washed for 1 hour at 37°C in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 minutes. Step 4: Filters are autoradiographed. Other conditions of high stringency that may be used are well known in the art. See, e.g., Ausubel et al., (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, NY; and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

[0043] Moderate stringency conditions can include the following: Step 1: Filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA. Step 2: Filters are hybridized for 18-20 hours at 55°C in the same solution with 5-20 x 10⁶ cpm ³²P-labeled probe added. Step 3: Filters are washed at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS, then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Step 4: Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency that may be used are well-known in the art. See, e.g., Ausubel et al., (eds.), 1993, CURRENT PROTOCOL IN MOLECULAR BIOLOGY, John Wiley and Sons, NY; and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

[0044] Low stringency can include: Step 1: Filters containing DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA. Step 2: Filters are hybridized for 18-20 hours at 40°C in the same solution with the addition of 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 x 10⁶ cpm ³²P-labeled probe. Step 3: Filters are washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Step 4: Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency that may be used are well known in the art (e.g., as employed for cross-species hybridizations). See, e.g., Ausubel et al., (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, NY; and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

Chimeric Peptides Including a JNK Inhibitor Domain and a Trafficking Domain

[0045] In another aspect the invention provides a chimeric peptide that includes a first and second domain. The first domain includes a trafficking sequence, while the second domain includes a JNK inhibitor sequence linked by a covalent bond, e.g. peptide bond, to the first domain. The first and second domains can occur in any order in the peptide, and the peptide can include one or more of each domain.

[0046] A trafficking sequence is any sequence of amino acids that directs a peptide in which it is present to a desired cellular destination. Thus, the trafficking sequence can direct the peptide across the plasma membrane, e.g., from outside the cell, through the plasma membrane, and into the cytoplasm. Alternatively, or in addition, the trafficking sequence can direct the peptide to a desired location within the cell, e.g., the nucleus, the ribosome, the ER, a lysosome, or peroxisome.

[0047] The trafficking peptide may be derived from a known membrane- translocating sequence. For example, the trafficking peptide may include sequences from the human immunodeficiency virus (HIV)1 TAT protein. This protein is described in, e.g., U.S. Patent Nos. 5,804,604 and 5,674,980, each incorporated herein by reference. The JNK inhibitor peptide may be linked to some or all of the entire 86 amino acids that make up the TAT protein. For example, a functionally effective fragment or portion of a TAT protein that has fewer than 86 amino acids, which exhibits uptake into cells, and optionally uptake into the cell nucleus, can be used. The fragment may include a peptide containing TAT residues 48-57, e.g. NH₂-GRKKRRQRRR-cooH [SEQ ID NO: 7] or a generic TAT sequence NH₂-X_n-RKKRRQRRR-X_n-cooH [SEQ ID NO: 9]. A TAT peptide that includes the region that mediates entry and uptake into cells can be further defined using known techniques. See, e.g., Franked et al, Proc. Natl. Acad. Sci, USA 86: 7397-7401 (1989).

[0048] The TAT sequence may be linked either to the N-terminal or the C-terminal end of JNK inhibitor sequence. A hinge of two proline residues may be added between the TAT and JNK inhibitor peptide to create the full fusion peptide. For example, L-amino acid fusion peptides maybe the L-TAT-IB1 peptide [SEQ ID NO:11],, or the generic L-TAT-IB peptide [SEQ ID NO:13]. Dretro-inverso fusion peptides may be the D-TAT-IB1 peptide [SEQ ID NO:14], or the generic D-TAT-IB peptide [SEQ ID NO:16]. The TAT peptide may be a D retro-inverso peptide having the sequence NH₂-X_n-RRR-QRRKKR-X_n-cooH [SEQ ID NO:10]. In SEQ ID NOs:5-6, 9-10, 13 and 16, the number of "X" residues is not limited to the one depicted, and may vary as described above.

[0049] The trafficking sequence can be a single (*i.e.*, continuous) amino acid sequence present in the TAT sequence. Alternatively it can be two or more amino acid sequences, which are present in TAT protein, but in the naturally-occurring protein are separated by other amino acid sequences. As used herein, TATprotein includes a naturally-occurring amino acid sequence that is the same as that of naturally-occurring TAT protein, or its functional equivalent protein or functionally equivalent fragments thereof (peptides). Such functional equivalent proteins or functionally equivalent fragments possess uptake activity into the cell and into the cell nucleus that is substantially similar to that of naturally-occurring TAT protein. TAT protein can be obtained from naturally-occurring sources or can be produced using genetic engineering techniques or chemical synthesis.

[0050] The amino acid sequence ofnaturally-occurring HIV TAT protein can be modified, for example, by addition, deletion and/or substitution of at least one amino acid present in the naturally-occurring TAT protein, to produce modified TAT protein (also referred to herein as TATprotein). Modified TAT protein or TAT peptide analogs with increased or decreased stability can be produced using known techniques. TAT proteins or peptides may include amino acid sequences that are substantially similar, although not identical, to that of naturally-occurring TATprotein orportions thereof. In addition, cholesterol or other lipid derivatives can be added to TAT protein to produce amodified TAT having increased membrane solubility.

[0051] Variants ofthe TAT protein can be designed to modulate intracellular localization of TAT-JNKinhibitorpeptide. When added exogenously, such variants are designed such that the ability of TAT to enter cells is retained (*i.e.*, the uptake ofthe variant TAT protein orpeptide into the cell is substantially similar to that of naturally-occurring HIV TAT). For example, alteration ofthe basic region thought to be important for nuclear localization (see, e.g., Dang and Lee,J. Bio!. Chem. 264: 18019-18023 (1989); Hauber et al., J. virol. 63: 1181-1187 (1989); Ruhen et al., J. Virol. 63: 1-8(1989)) can result inacytoplasmic location orpartially cytoplasmic location ofTAT, and therefore, ofthe JNK inhibitor peptide. Alternatively, a sequence for binding a cytoplasmic or any other component or compartment (e.g., endoplasmic reticule, mitochondria,

gloom apparatus, lysosomal vesicles,) can be introduced into TAT in order to retain TAT and the JNK inhibitor peptide in the cytoplasm or any other compartment to confer regulation upon uptake of TAT and the JNK inhibitor peptide.

[0052] Other sources forthe trafficking peptide include, e.g., VP22 (described in,e.g., WO 97/05265; Elliott and O'Hare, Ce/188: 223-233 (1997)), ornon-viral proteins (Jackson et al, Proc. Natl. Acad. Sci. USA89: 10691-10695 (1992)).

[0053] The JNK inhibitor sequence and the trafficking sequence can be linked by chemical coupling in any suitable manner known in the art. Many known chemical cross-linking methods are non-specific, *i.e.*; they do not direct the point of coupling to any particular site on the transport polypeptide or cargo macromolecule. As a result, use of non-specific cross-linking agents may attack functional sitesorsterically block active sites, rendering the conjugated proteins biologically inactive.

[0054] One way to increasing coupling specificity is to directly chemical coupling to a functional group found only once or a few times in one or both of the polypeptides to be crosslinked. For example, in many proteins, cysteine, which is the only protein amino acid containing a thiol group, occurs only a few times. Also, for example, if a polypeptide contains no lysine residues, a cross-linking reagent specific for primary amines will be selective for the amino terminus of that polypeptide. Successful utilization of this approach to increase coupling specificity requires that the polypeptide have the suitably rare and reactive residues in areas of the molecule that may be altered without loss of the molecule's biological activity.

[0055] Cysteine residues may be replaced when they occur in parts of a polypeptide sequence where their participation in a cross-linking reaction would otherwise likely interfere with biological activity. When a cysteine residue is replaced, it is typically desirable to minimize resulting changes in polypeptide folding. Changes in polypeptide folding are minimized

when the replacement is chemically and sterically similar to cysteine. For these reasons, serine is preferred as a replacement for cysteine. As demonstrated in the examples below, a cysteine residue may be introduced into a polypeptide's amino acid sequence for cross-linking purposes. When a cysteine residue is introduced, introduction at or near the amino or carboxy terminus is preferred. Conventional methods are available for such amino acid sequence modifications,

whether the polypeptide of interest is produced by chemical synthesis or expression of recombinant DNA. **[0056]** Coupling of the two constituents can be accomplished via a coupling or conjugating agent. There are several intermolecular cross-linking reagents which can be utilized, *See for example*, Means and Feeney, CHEMICAL MODIFICATION OF PROTEINS, Holden-Day, 1974, pp. 39-43. Among these reagents are, for example, J-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) or N, N- (1,3-phenylene) bismaleimide (both of which are highly specific for sulfhydryl groups and form irreversible linkages); N, N'-ethylene-bis- (iodoacetamide) or other such reagent having 6 to 11 carbon methylene bridges (which relatively specific for sulfhydryl groups); and 1,5-difluoro-2, 4-dinitrobenzene (which forms irreversible linkages with amino and tyrosine groups). Other cross-linking reagents useful for this purpose include: p,p'-difluoro-m,m'-dinitrodiphenylsulfone (which forms irreversible cross-linkages with amino and phenolic groups); dimethyl adipimidate (which is specific for amino groups); phenol-1,4-disulfonylchloride (which reacts principally with amino groups); hexamethylenediisocyanate or diisothiocyanate, or azophenyl-p-diisocyanate (which reacts principally with amino groups); glutaraldehyde (which reacts with several different side chains) and disdiazobenzidine (which reacts primarily with tyrosine and histidine).

[0057] Cross-linking reagents may be homobifunctional, i.e., having two functional groups that undergo the same reaction. A preferred homobifunctional cross-linking reagent is bismaleimido-hexane ("BMH"). BMH contains two maleimide functional groups, which react specifically with sulfhydryl-containing compounds under mild conditions (pH 6.5-7.7). The two maleimide groups are connected by a hydrocarbon chain. Therefore, BMH is useful for irreversible cross-linking of polypeptides that contain cysteine residues.

[0058] Cross-linking reagents may also be heterobifunctional. Heterobifunctional cross-linking agents have two different functional groups, for example an amine-reactive group and a thiol-reactive group, that will cross-link two proteins having free amines and thiols, respectively. Examples of heterobifunctional cross-linking agents are succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate ("SMCC"), m-maleimidobenzoyl-N-hydroxysuccinimide ester ("MBS"), and succinimide 4-(p-maleimidophenyl) butyrate ("SMPB"), an extended chain analog of MBS. The succinimidyl group of these cross-linkers reacts with a primary amine, and the thiol-reactive maleimide forms a covalent bond with the thiol of a cysteine residue.

[0059] Cross-linking reagents often have low solubility in water. A hydrophilic moiety, such as a sulfonate group, may be added to the cross-linking reagent to improve its water solubility. Sulfo-MBS and sulfo-SMCC are examples of cross-linking reagents modified for water solubility.

[0060] Many cross-linking reagents yield a conjugate that is essentially non-cleavable under cellular conditions. However, some cross-linking reagents contain a covalent bond, such as a disulfide, that is cleavable under cellular conditions. For example, Traut's reagent, dithiobis (succinimidylpropionate) ("DSP"), and N-succinimidyl 3-(2-pyridyldithio) propionate ("SPDP") are well-known cleavable cross-linkers. The use of a cleavable cross-linking reagent permits the cargo moiety to separate from the transport polypeptide after delivery into the target cell. Direct disulfide linkage may also be useful.

[0061] Numerous cross-linking reagents, including the ones discussed above, are commercially available. Detailed instructions for their use are readily available from the commercial suppliers. A general reference on protein cross-linking and conjugate preparation is: Wong, CHEMISTRY OF PROTEIN CONJUGATION AND CROSS-LINKING, CRC Press (1991).

[0062] Chemical cross-linking may include the use of spacer arms. Spacer arms provide intramolecular flexibility or adjust intramolecular distances between conjugated moieties and thereby may help preserve biological activity. A spacer arm may be in the form of a polypeptide moiety that includes spacer amino acids, e.g. proline. Alternatively, a spacer arm may be part of the cross-linking reagent, such as in "long-chain SPDP" (Pierce Chem. Co" Rockford, IL., cat. No. 21651 H).

[0063] Alternatively, the chimeric peptide can be produced as a fusion peptide that includes the trafficking sequence and the JNK inhibitor sequence which can conveniently be expressed in known suitable host cells. Fusion peptides, as described herein, can be formed and used in ways analogous to or readily adaptable from standard recombinant DNA techniques, as described above.

PRODUCTION OF ANTIBODIES SPECIFIC FOR JNK INHIBITOR PEPTIDES

[0064] Chimeric peptides including the JNK inhibitor peptides, which are equal or less than 25 amino acids in length and comprises SEQ ID NO: 1, 3, 5 or 6 or homologues thereof having at least 80% identity to SEQ ID NO: 1, 3, 5 or 6 may be utilized as immunogens to generate antibodies that immunospecifically bind these peptide components. Such antibodies include, e.g., polyclonal, monoclonal, chimeric, single chain, Fab fragments and a Fab expression library.

Antibodies to human peptides are disclosed. Fragments of the JNK inhibitor peptides may be used as immunogens for antibody production. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to a JNK inhibitor peptide, or derivative, fragment, analog or homolog thereof.

[0065] For the production of polyclonal antibodies, various host animals may be immunized by injection with the native peptide, or a synthetic variant thereof, or a derivative of the foregoing. Various adjuvants may be used to increase the immunological response and include, but are not limited to, Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.) and human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium*

parvum.

[0066] For preparation of monoclonal antibodies directed towards a JNK inhibitor peptide, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see, Kohler and Milstein, 1975, *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see, Kozbor, et al., 1983, *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see, Cole, et al., 1985, In: *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by the use of human hybridomas (see, Cote, et al., 1983, *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see, Cole, et al., 1985, In: *Monoclonal Antibodies and Cancer Therapy* (Alan R. Liss, Inc., pp. 77-96).

[0067] According to the invention, techniques can be adapted for the production of single-chain antibodies specific to a JNK inhibitor peptide (see, e.g., U.S. Patent No. 4,946,778). In addition, methodologies can be adapted for the construction of Fab expression libraries (see, e.g., Huse, et al., 1989, *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a JNK inhibitor peptide or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See e.g., U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotype to a JNK inhibitor peptide may be produced by techniques known in the art including, e.g., (i) an F(ab')₂ fragment produced by pepsin digestion of an antibody molecule; (ii) an Fab fragment generated by reducing the disulfide bridges of an F(ab')₂ fragment; (iii) an Fab fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) Fv fragments.

[0068] Methodologies for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. Selection of antibodies that are specific to a particular domain of a JNK inhibitor peptide is facilitated by generation of hybridomas that bind to the fragment of a JNK inhibitor peptide possessing such a domain. Antibodies that are specific for a domain within a JNK

inhibitor peptide, or derivative, fragments, analogs or homologs thereof, are also provided herein.

[0069] The anti-JNK inhibitor peptide antibodies may be used in methods known within the art relating to the localization and/or quantitation of a JNK inhibitor peptide (e.g., for use in measuring levels of the peptide within appropriate physiological samples, for use in diagnostic methods, for use in imaging the peptide, and the like). Antibodies for the JNK inhibitor peptides, or derivatives, fragments, analogs or homologs thereof that contain the antibody derived binding domain, may be utilized as pharmacologically active compounds [hereinafter "Therapeutics"].

PEPTIDES FOR USE IN METHODS OF TREATING OR PREVENTING DISORDERS ASSOCIATED UNDESIRE JNK

ACTIVITY

[0070] Also included in the invention is the use of the claimed peptide in methods of treating cell-proliferative disorders associated with JNK activation in a subject by administering to a subject a biologically-active therapeutic compound (hereinafter "Therapeutic"). The subject can be e.g., any mammal, e.g., a human, a primate, mouse, rat, dog, cat, cow, horse, pig. The Therapeutics include any one of the claimed JNK inhibitor peptides and claimed homologs thereof

[0071] The term "therapeutically effective" means that the amount of inhibitor peptide, for example, which is used, is of sufficient quantity to ameliorate the JNK associated disorder. The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations that often appear to differ morphologically and functionally from the surrounding tissue. For example, the method may be useful in treating malignancies of the various organ systems, in which activation of JNK has often been demonstrated, e.g., lung, breast, lymphoid,

gastrointestinal, and genito-urinary tracts as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Cancers with Bcr-Abl oncogenic transformations that clearly require activation of JNK are also included.

[0072] The peptides are also useful in treating non-malignant or immunological-related cell-proliferative diseases such as psoriasis, pemphigus vulgaris, Behcet's syndrome, acute respiratory distress syndrome (ARDS), ischemic heart disease, post-dialysis syndrome, leukemia, rheumatoid arthritis, acquired immune deficiency syndrome, vasculitis, septic shock and other types of acute inflammation, and lipid histiocytosis. Especially preferred are immunopathological disorders. Essentially, any disorder, which is etiologically linked to JNK kinase activity, would be considered susceptible to treatment.

[0073] Treatment includes administration of a reagent that modulates JNK kinase activity. The term "modulate" includes the suppression of expression of JNK when it is over-expressed. It also includes suppression of phosphorylation of c-jun or ATF2, for example, by using a peptide of anyone of SEQ ID NOs: 1, 3, 5 or 6 and SEQ ID NOs: 11, 13, 14 or 16 as a competitive inhibitor of the natural c-jun and ATF2 binding site in a cell.

Thus also includes suppression of hetero- and homo-meric complexes of transcription factors made up of c-jun, or ATF2. When a cell proliferative disorder is associated with JNK

overexpression, such suppressive JNK inhibitor peptides can be introduced to a cell. In some instances, "modulate" may include the increase of JNK expression, for example by use of an IB peptide-specific antibody that blocks the binding of an IB-peptide to JNK, thus preventing JNK inhibition by the IB-related peptide.

[0074] The claimed JNK inhibitor, peptides, or fusion peptides of the invention can be formulated in pharmaceutical compositions. These compositions may comprise, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal or patch routes.

[0075] Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

[0076] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

[0077] Whether it is a polypeptide, peptide, or nucleic acid molecule, other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in REMINGTON'S PHARMACEUTICAL SCIENCES, 16th edition, Osol, A. (ed), 1980.

[0078] Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibody or cell specific ligands. Targeting may be desirable for a variety of reasons; for example if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

IMMUNOASSAYS

[0079] The peptides of the present invention may be utilized in assays (e.g., immunoassays) to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders characterized by aberrant levels of JNK, or a JNK inhibitor peptide, or monitor the treatment thereof. An "aberrant level" means an increased or decreased level in a sample relative

to that present in an analogous sample from an unaffected part of the body, or from a subject not having the disorder. The immunoassay may be performed by a method comprising contacting a sample derived from a patient with an antibody under conditions such that immunospecific-binding may occur, and subsequently detecting or measuring the amount of any immunospecific-binding by the antibody. An antibody specific for a

JNK inhibitor peptide may be used to analyze a tissue or serum sample from a patient for the presence of JNK or a JNK inhibitor peptide; wherein an aberrant level of JNK or a JNK inhibitor peptide is indicative of a diseased condition. The immunoassays that may be utilized include, but are not limited to, competitive and non-competitive assay systems using

techniques such as Western Blots, radioimmunoassays (RIA), enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, fluorescent immunoassays, complement-fixation assays, immunoradiometric assays, and protein-A immunoassays, etc.

SPECIFIC EXAMPLES

Example 1: Identification of JNK Inhibitor Peptides

[0080] Amino acid sequences important for efficient interaction with JNK were identified by sequence alignments between known JBDs. Sequence comparison between the JBDs of IB1 [SEQ ID NO:17], IB2 [SEQ ID NO:18], c-Jun [SEQ ID NO:19] and ATF2 [SEQ ID NO:20] defined a weakly conserved 8 amino acid sequence (FIG. 1A). Since the JBDs of IB1 and IB2 are approximately 100 fold as efficient as c-Jun or ATF2 in binding JNK (Dickens et al. Science 277: 693 (1997)), it was reasoned that conserved residues between IB1 and IB2 must be important to confer maximal binding. The comparison between the JBDs of IB1 and IB2 defined two blocks of seven and three amino acids that are highly conserved between the two sequences. These two blocks are contained within a peptide sequence of 23 amino acids in IB1 [SEQ ID NO:1] and 21 amino acid IB2 [SEQ ID NO:2]. These sequences are shown in FIG. 1B, dashes in the IB2 sequence indicate a gap in the sequence in order to align the conserved residues.

Example 2: Preparation of JNK Inhibitor Fusion Proteins

[0081] JNK inhibitor fusion proteins were synthesized by covalently linking the C-terminal end of JBD₂₃ or the 21 amino acid sequence derived from the JBD of IB2 (JBD₂₁) to a N-terminal 10 amino acid long carrier peptide derived from the HIV-TAT₄₈₋₅₇ (Vives et al., J. Biol. Chem. 272: 16010 (1997)) via a spacer consisting of two proline residues. This spacer was used to allow for maximal flexibility and prevent unwanted secondary structural changes. As shown in FIG. 1C, these preparations were designated L-TAT [SEQ ID NO:7], L-TAT-IB1 [SEQ ID NO:11] and L-TAT-IB2 [SEQ ID NO:12], respectively. All-D retro-inverso peptides TAT-fusion peptides were also synthesized and were designated D-TAT [SEQ ID NO:8] and D-TAT-IB1 [SEQ ID NO: 14], respectively. All D and L peptides were produced by classical F-mock synthesis and further analysed by Mass Spectrometry. They were finally purified by HPLC. To determine the effects of the proline spacer, two types of TAT peptide were produced one with and one without two prolines. The addition of the two prolines did not appear to modify the entry or the localization of the TAT peptide inside cells.

[0082] Generic peptides showing the conserved amino acid residues are given in FIG. 2. An "X" indicates any amino acid. The number of Xs in a given peptide is not limited to the one depicted, and may vary. See above for a more detailed description of the generic sequences.

Example 3: Inhibition of β Cell Death By JBD₂₃

[0083] Effects of the 23 a.a. long JBD sequence of IB1 on JNK biological activities were then studied. The 23 a.a. sequence was linked N-terminal to the Green Fluorescent Protein (GFP-JBD₂₃ construct), and the effect of this construct on pancreatic β -cell apoptosis induced by IL-1 β was evaluated. See FIG. 3. This mode of apoptosis was previously shown to be blocked by transfection with JBD₁₋₂₈₀, whereas specific inhibitors of ERK1/2 or p38 did not protect. See Ammendrup et al, *supra*.

[0084] Oligonucleotides corresponding to the 23 amino acid sequence (JBD₂₃; FIG 1B) and a sequence mutated at the fully conserved regions (JBD_{23mut}) were synthesized and directionally inserted into the EcoRI and SalI sites of the pEGFP-N1 vector encoding the Green Fluorescent Protein (GFP) (from Clontech). Insulin producing β TC-3 cells were cultured in RPMI 1640 medium supplemented with 10% Fetal Calf Serum, 100 μ g/mL Streptomycin, 100 units/mL Penicillin and 2 mM Glutamine. Insulin producing β TC-3 cells were transfected with the indicated vectors and IL-1 β (10 ng/mL) was added to the cell culture medium. The number of apoptotic cells were counted at 48 hours after the addition of IL-1 β using an inverted fluorescence microscope. Apoptotic cells were discriminated from normal cells by the characteristic "blebbing out" of the cytoplasm were counted after two days.

[0085] As indicated in FIG. 3, GFP is Green Fluorescent protein expression vector used as a control; JBD23 is the vector expressing a chimeric GFP linked to the 23 a.a. sequence from the JBD of IB1; JBD23Mut is the same vector as GFP-JBD23, but with a JBD mutated at four conserved residues shown as FIG 1B; and JBD280 is the GFP vector linked to the entire JBD (a.a. 1-280). The GFP-JBD₂₃ expressing construct prevented IL-1 β induced pancreatic β -cell apoptosis as efficiently as the entire JBD₁₋₂₈₀ (FIG. 3, JBD23/IL-1 compared to JBD280/IL-1). As additional controls, sequences mutated at fully conserved IB1 residues had greatly decreased ability to prevent apoptosis (FIG. 3, JBD23Mut/IL-1).

Example 4: Cellular Import Of TAT-IB1 And TAT-IB2 Peptides

[0086] The ability of the L- and D-enantiomeric forms of TAT, TAT-IB1 and TAT-IB2 peptides ("TAT-IB peptides") to enter cells were evaluated.

[0087] L-TAT, D-TAT, L-TAT-IB1, L-TAT-IB2 and D-TAT-IB1 peptides [SEQ ID NOs: 7, 8, 11, 12 and 14, respectively] were labeled by N-terminal addition of a glycine residue conjugated to fluorescein. Labeled peptides (1 μ M) were added to β TC-3 cell cultures, which were maintained as described in Example 3. At predetermined times, cells were washed with PBS and fixed for five minutes in ice-cold methanol-acetone (1:1) before being examined under a fluorescence microscope. Fluorescein-labeled BSA (1 μ M, 12 moles/mole BSA) was used as a control. Results demonstrated that all the above fluorescein labeled peptides had efficiently and rapidly (less than five minutes) entered cells once added to the culture medium. Conversely, fluorescein labeled bovine serum albumin (1 μ M BSA, 12 moles fluorescein/mole BSA) did not enter the cells.

[0088] A time course study indicated that the intensity of the fluorescent signal for the L-enantiomeric peptides decreased by 70% following a 24 hours period. Little to no signal was present at 48 hours. In contrast, D-TAT and D-TAT-IB1 were extremely stable inside the cells. Fluorescent signals from these all-D retro-inverso peptides were still very strong 1 week later, and the signal was only slightly diminished at 2 weeks post treatment.

Example 5: *In Vitro* Inhibition Of c-JUN, ATF2 and Elk1 Phosphorylation

[0089] The effects of the peptides on JNKs-mediated phosphorylation of their target transcription factors were investigated *in vitro*. Recombinant and nonactivated JNK1, JNK2 and JNK3 were produced using a TRANSCRIPTION AND TRANSLATION rabbit reticulocyte lysate kit (Promega) and used in solid phase kinase assays with c-Jun, ATF2 and Elk1, either alone or fused to glutathione-S-transferase (GST), as substrates. Dose response studies were performed wherein L-TAT, L-TAT-IB1 or L-TAT-IB2 peptides (0-25 μ M) were mixed with the recombinant JNK1, JNK2, or JNK3 kinases in reaction buffer (20 mM Tris-acetate, 1mM EGTA, 10mM p-nitrophenyl-phosphate (pNPP), 5 mM sodium pyrophosphate, 10 mM β -glycerophosphate, 1mM dithiothreitol) for 20 minutes. The kinase reactions were then initiated by the addition of 10 mM $MgCl_2$ and 5 μ Ci ^{33}P - γ -dATP and 1 μ g of either GST-Jun (a.a. 1-89), GST-ATF2 (a.a. 1-96) or GST-ELK1 (a.a. 307-428). GST-fusion proteins were purchased from Stratagene (La Jolla, CA). Ten μ L of glutathione-agarose beads were also added to the mixture. Reaction products were then separated by SDS-PAGE on a denaturing 10 % polyacrylamide gel. Gels were dried and subsequently exposed to X-ray films (Kodak). Nearly complete inhibition of c-Jun, ATF2 and Elk1 phosphorylation by JNKs was observed at TAT-IB peptide doses as low as 2.5 μ M. However, a marked exception was the absence of TAT-IB inhibition of JNK3 phosphorylation of Elk1. Overall, the TAT-IB1 peptide appeared slightly superior to TAT-IB2 in inhibiting JNK family phosphorylation of their target transcription factors. (See, FIG. 4A).

[0090] The ability of D-TAT, D-TAT-IB1 and L-TAT-IB1 peptides (0-250 μ M dosage study) to inhibit GST-Jun (a.a. 1-73) phosphorylation by recombinant JNK1, JNK2, and JNK3 by were analyzed as described above. Overall, D-TAT-IB1 1 peptide decreased JNK-mediated phosphorylation of c-Jun, but at levels approximately 10-20 fold less efficiently than L-TAT-IB1. (See, FIG. 4B).

Example 6: Inhibition of c-JUN Phosphorylation By Activated JNKs

[0091] The effects of the L-TAT, L-TAT-IB1 or L-TAT-IB2 peptides on JNKs activated by stressful stimuli were evaluated using GST-Jun to pull down JNKs from UV-light irradiated HeLa cells or IL-1 β treated β TC cells. β TC cells were cultured as described above. HeLa cells were cultured in DMEM medium supplemented with 10 % Fetal Calf Serum, 100 μ g/mL Streptomycin, 100 units/ml Penicillin and 2 mM Glutamine. One hour prior to being used for cell extract preparation, β TC cells were activated with IL-1 β as described above, whereas HeLa cells were activated by UV-light (20 J/m²). Cell extracts were prepared from control, UV-light irradiated HeLa cells and IL-1 β treated β TC-3 cells by scraping the cell cultures in lysis buffer (20 mM Tris-acetate, 1mM EGTA, 1% Triton X-100, 10 mM p-nitrophenyl-phosphate, 5 mM sodium pyrophosphate, 10 mM β -glycerophosphate, 1 mM dithiothreitol). Debris was removed by centrifugation for five minutes at 15,000 rpm in an SS-34 Beckman rotor. One-hundred μ g extracts were incubated for one hour at room temperature with one μ g GST-jun (amino acids 1-89) and 10 μ L of glutathione-agarose beads (Sigma). Following four washes with the scraping buffer, the beads were resuspended in the same buffer supplemented with L-TAT, L-TAT-IB1 or L-TAT-IB2 peptides (25 μ M) for 20 minutes. Kinase reactions were then initiated by the addition of 10 mM $MgCl_2$ and 5 μ Ci ^{33}P - γ -dATP and incubated for 30 minutes at 30°C. Reaction products were then separated by SDS-PAGE on a denaturing 10 % polyacrylamide gel. Gels were dried and subsequently exposed to X-ray films (Kodak). The TAT-IB peptides efficiently prevented phosphorylation of c-Jun by activated JNKs in these experiments.. (See, FIG. 6).

Example 7: In vivo Inhibition of c-JUN Phosphorylation By TAT-IB Peptides

[0092] To determine whether the cell-permeable peptides could block JNK signaling *in vivo*, we used a heterologous GAL4 system. HeLa cells, cultured as described above, were co-transfected with the 5xGAL-LUC reporter vector together with the GAL-Jun expression construct (Stratagene) comprising the activation domain of c-Jun (amino acids 1-89) linked to the GAL4 DNA-binding domain. Activation of JNK was achieved by the co-transfection of vectors expressing the directly upstream kinases MKK4 and MKK7 (See, Whitmarsh et al., Science 285: 1573 (1999)). Briefly, 3×10^5 cells were transfected with the plasmids in 3.5-cm dishes using DOTAP (Boehringer Mannheim) following instructions from the manufacture. For experiments involving GAL-Jun, 20 ng of the plasmid was transfected with 1 μ g of the reporter plasmid pFR-Luc (Stratagene) and 0.5 μ g of either MKK4 or MKK7 expressing plasmids. Three hours following transfection, cell media were changed and TAT, TAT-IB 1, and TAT-IB2 peptides (1 μ M) were added. The luciferase activities were measured 16 hours later using the "Dual Reporter System" from Promega after normalization to protein content. As shown in FIG. 5, addition of both the TAT-IB1 and TAT-IB2 peptides blocked activation of c-Jun following MKK4 and MKK7 mediated activation of JNK. Because HeLa cells express both JNK1 and JNK2 isoforms but not JNK3, we transfected cells with JNK3. Again, the two TAT-IBpeptides inhibited JNK2 mediated activation of c-Jun.

Example 8: Inhibition Of IL-1 β Induced Pancreatic β -Cell Death By TAT-IB Peptides

[0093] We investigated the effects of the L-TAT-IB peptides on the promotion of pancreatic β -cell apoptosis elicited by IL-1 β . β TC-3 cell cultures were incubated for 30 minutes with 1 μ M of either L-TAT-IB1 or L-TAT-IB2 peptides followed by 10 ng/mL of IL-1 β . A second addition of peptide (1 μ M) was performed 24 hours later. Apoptotic cells were counted after two days of incubation with IL-1 β using Propidium Iodide (red stained cell are dead cells) and Hoechst 33342 (blue stained cell are cells with intact plasma membrane) nuclear staining. As shown in FIG. 5, addition of the TAT-IBpeptides inhibited IL-1 β -induced apoptosis of β TC-3 cells cultured in the presence of IL-1 β for two days.

[0094] Long term inhibition of IL-1 β induced cells death was examined by treating β TC-3 cells as described above, except that incubation of the cells with the peptides and IL-1 β was sustained for 12 days. Additional peptides (1 μ M) were added each day and additional IL-1 β (10 ng/mL) was added every 2 days. The TAT-IB1 peptide confers strong protection against apoptosis in these conditions. Taken together, these experiments establish that TAT-IBpeptides are biologically active molecules able to prevent the effects of JNK signaling on cell fate.

Example 9: Synthesis of an All-D-retro-inverso Peptides

[0095] Peptides of the invention may be all-D amino acid peptides synthesized in reverse to prevent natural proteolysis (*i.e.*, all-D-retro-inverso peptides). An all-D retro-inverso peptide of the invention would provide a peptide with functional properties similar to the native peptide, wherein the side groups of the component amino acids would correspond to the native peptide alignment, but would retain a protease resistant backbone.

[0096] Retro-inverse peptides of the invention are analogs synthesized using D-amino acids by attaching the amino acids in a peptide chain such that the sequence of amino acids in the retro-inverso peptide analog is exactly opposite of that in the selected peptide which serves as the model. To illustrate, if the naturally occurring TAT protein (formed of L-amino acids) has the sequence GRKKRRQRRR [SEQ ID NO:7], the retro-inverso peptide analog of this peptide (formed of D-amino acids) would have the sequence RRRQRRKKRG [SEQ ID NO:8]. The procedures for synthesizing a chain of D-amino acids to form the retro-inverso peptides are known in the art. See, *e.g.*, Jameson et al., Nature, 368, 744-746 (1994); Brady et al., Nature, 368,692-693 (1994); Guichard et al., J. Med. Chem. 39,2030-2039 (1996). Specifically, the retro- peptides were produced by classical F-mock synthesis and further analysed by Mass Spectrometry. They were finally purified by HPLC.

[0097] Since an inherent problem with native peptides is degradation by natural proteases and inherent immunogenicity, the heterobivalent or heteromultivalent compounds of this invention will be prepared to include the "retro-inverso isomer" of the desired peptide. Protecting the peptide from natural proteolysis should therefore increase the effectiveness of the specific heterobivalent or heteromultivalent compound, both by prolonging half-life and decreasing the extent of the immune response aimed at actively destroying the peptides.

Example 10: Long term biological activity of all-D-retro-inverso IB Peptides

[0098] Long term biological activity is predicted for the D-TAT-IB retro-inverso containing peptide heteroconjugate when compared to the native L-amino acid analog owing to protection of the D-TAT-IB peptide from degradation by native proteases, as shown in Example 5.

[0099] Inhibition of IL-1 β induced pancreatic β -cell death by the D-TAT-IB1 1 peptide was analyzed. As shown in FIG. 10, β TC-3 cells were incubated as described above for 30 minutes with one single addition of the indicated peptides

(1 μ M), then IL-1 β (10 ng/ml) was added. Apoptotic cells were then counted after two days of incubation with IL-1 β by use of Propidium Iodide and Hoechst 33342 nuclear staining. A minimum of 1,000 cells were counted for each experiment. Standard Error of the Means (SEM) are indicated, n=5. The D-TAT-IB1 peptide decreased IL-1 induced apoptosis to a similar extent as L-TAT-IB1 peptides (compare FIG. 5 and FIG. 10).

[0100] Long term inhibition of IL-1 β induced cell-death by the D-TAT-IB1 peptide was also analyzed. β TC-3 cells were incubated as above for 30 minutes with one single addition of the indicated peptides (1 μ M), then IL-1 β (10 ng/ml) was added, followed by addition of the cytokine every two days. Apoptotic cells were then counted after 15 days of incubation with IL-1 β by use of Propidium Iodide and Hoechst 33342 nuclear staining. Note that one single addition of the L-TAT-IB peptide does not confer long-term protection. A minimum of 1,000 cells were counted for each experiment. Standard Error of the Means (SEM) are indicated, n=5. Results are shown in FIG.9. D-TAT-IB1, but not L-TAT-IB 1, was able to confer long term (15 day) protection.

Example 11: Inhibition Of Irradiation Induced Pancreatic β -Cell Death By TAT-IBPeptides

[0101] JNK is also activated by ionizing radiation. To determine whether TAT-IBpeptides would provide protection against radiation-induced JNK damage, "WiDr" cells were irradiated (30Gy) in presence or absence of D-TAT, L-TAT-IB1 or D-TAT-IB1 peptides (1 μ M added 30 minutes before irradiation), as indicated in FIG. 10. Control cells (CTRL) were not irradiated. Cells were analyzed 48 hours later by mean of PI and Hoechst 33342 staining, as described above. n=3, SEM are indicated. L-TAT-IB1 and D-TAT-IB1 peptides were both able to prevent irradiation induced apoptosis in this human colon cancer cell line.

Example 12: Radioprotection to Ionizing Radiation By TAT-IB Peptides

[0102] To determine the radioprotective effects of the TAT-IB peptides, C57 B1/6 mice (2 to 3 months old) were irradiated with a Phillips RT 250 R-ray at a dose rate of 0.74 Gy/min (17 mA, 0.5 mm Cu filter). Thirty minutes prior to irradiation, the animals were injected i.p. with either the TAT, L-TAT-IB1 and D-TAT-IB1 peptides (30 μ l of a 1mM solution). Briefly, mice were irradiated as follows: mice were placed in small plastic boxes with the head lying outside the box. The animals were placed on their back under the irradiator, and their neck fixed in a small plastic tunnel to maintain their head in a correct position. The body was protected with lead. Prior to irradiation mice were maintained on standard pellet mouse chow, however post irradiation mice were fed with a semi-liquid food that was renewed each day.

[0103] The reaction of the lip mucosa was then scored by 2 independent observers according to the scoring system developed by Parkins *et al.* (Parkins et al, Radiotherapy & Oncology, 1: 165-173, 1983), in which the erythema status as well as the presence of edema, desquamation and exudation was quoted. Additionally, animals were weighed before each recording of their erythema/edema status.

[0104] FIG. 12A: illustrated the weight of the mice following irradiation. Values are reported to the initial weight of the mice that was set to 100. CTRL: control mice injected with 30 μ l of a saline solution. n=2 for each values reported, S.D. are indicated. x values are days

[0105] FIG. 12B is illustrative of the erythema/edema scoring following irradiation. The edema and erythema status of the ventral lip of the same mice as in FIG. 12A was quantified. n=2 for each value reported. x values are days

[0106] The results of these experiments indicate that the TAT-IB Peptides can protect against weight loss and erythema/edema associated with ionizing radiation.

Example 13: Suppression of JNK Transcription Factors by L-TAT-IB1 peptides

[0107] Gel retardation assays were carried out with an AP-1 doubled labeled probe (5'- CGC TTG ATG AGT CAG CCG GAA-3'. HeLa cell nuclear extracts that were treated or not for one hour with 5 ng/ml TNF- α , as indicated. TAT and L-TAT-IB1 peptides were added 30 minutes before TNF- α . Only the part of the gel with the specific AP-1 DNA complex (as demonstrated by competition experiments with non-labeled specific and non-specific competitors) is shown. L-TAT-IB1 peptides decrease the formation of the AP-1 DNA binding complex in the presence of TNF- α . (See, FIG 11).

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Claims

1. A chimeric peptide comprising a first domain and a second domain linked by a covalent bond, the first domain comprising a trafficking sequence directing the chimeric peptide across the plasma membrane and/or to a desired cellular location, and the second domain comprising a JNK inhibitor sequence, wherein the JNK inhibitor peptide is equal or less than 25 amino acids in length and comprises SEQ ID NO: 1, 3, 5 or 6 or homologues thereof having at least 80% identity to SEQ ID NO: 1, 3, 5 or 6.
2. The peptide according to claim 1, wherein the trafficking sequence augments cellular uptake of the peptide.
3. The peptide according to claim 1, wherein the trafficking sequence directs nuclear localization of the peptide.
4. The peptide according to claim 1, wherein the trafficking sequence comprises the amino acid sequence of a human immunodeficiency virus TAT polypeptide.
5. The peptide according to claim 1, wherein the JNK inhibitor sequence binds JNK.
6. The peptide according to claim 1, wherein the JNK inhibitor sequence inhibits the activation of at least one JNK targeted transcription factor.
7. The peptide according to claim 6, wherein the JNK targeted transcription factor is selected from the group consisting of C-Jun, ATF2, and Elk1.
8. The peptide according to claim 1, wherein the JNK inhibitor sequence alters the JNK induced effects when introduced into a JNK expressing cell.
9. The peptide according to claim 8, wherein the JNK induced effect is selected from the group consisting of restenosis, oncogenic transformation, maturation and differentiation of immune cells, ischemia, reperfusion, hypoxia, hypothermia, hyperthermia, apoptosis and response to stressful stimuli.
10. The peptide according to claim 1, wherein said peptide comprises the amino acid sequence of SEQ ID NO: 11, 13, 14 or 16.
11. A pharmaceutical composition comprising a peptide according to any of claims 1-10, and a pharmaceutical carrier.
12. Use of a peptide according to any of claims 1-10, in the manufacture of a medicament for treating or preventing a pathophysiology associated with activation of JNK in a subject, wherein the pathophysiology is selected from malignancies including malignancies of the lung, breast, lymphoid, gastrointestinal, and genitor-urinary tract, leukemia, adenocarcinomas, including colon cancers, renal-cell carcinoma, prostate cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, and cancers with Bcr-Abl oncogenic transformation, and non-malignant or immunologically-related cell proliferative diseases selected from psoriasis, restenosis, vasculitis, pemphigus vulgaris, Behcet's syndrome, rheumatoid arthritis, acute respiratory distress syndrome (ARDS), ischemic heart disease, post-dialysis syndrome, acquired immunodeficiency syndrome, lipid histiocytosis, septic shock, diabetes, and pathophysiologies induced by ionizing radiation, free radicals, DNA damaging agents or UV light.
13. Peptide according to any of claims 1-10, for use in a method of treating or preventing a pathophysiology associated with activation of JNK in a subject, wherein the pathophysiology is selected from malignancies including malignancies of the lung, breast, lymphoid, gastrointestinal, and genitor-urinary tract, leukemia, adenocarcinomas, including colon cancers, renal-cell carcinoma, prostate cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, and cancers with Bcr-Abl oncogenic transformation, and non-malignant or immunologically-related cell proliferative diseases selected from psoriasis, restenosis, vas-

culitis, pemphigus vulgaris, Behcet's syndrome, rheumatoid arthritis, acute respiratory distress syndrome (ARDS), ischemic heart disease, post-dialysis syndrome, acquired immunodeficiency syndrome, lipid histiocytosis, septic shock, diabetes, and pathophysiologies induced by ionizing radiation, free radicals, DNA damaging agents or UV light.

14. Use according to claim 12 or the peptide for use according to claim 13, wherein the peptide blocks activation of at least one JNK targeted transcription factor.
15. Use or the peptide for use according to claim 14, wherein the JNK targeted transcription factor is selected from the group consisting of C-Jun, ATF2, and Elk1.
16. Use or the peptide for use according to claim 14, wherein the JNK targeted transcription factor is C-Jun.
17. Use or the peptide for use according to any of claims 12 to 16, wherein the medicament is suitable for delivery by any one administration route selected from the group consisting of intraperitoneal, subcutaneous, intramuscular, nasal, intravenous, oral and patch delivery.

Patentansprüche

1. Chimäres Peptid, das eine erste Domäne und eine zweite Domäne umfasst, die durch eine kovalente Bindung verbunden sind, wobei die erste Domäne eine Transportsequenz umfasst, die das chimäre Peptid durch die Plasmamembran hindurch und/oder an einen gewünschten zellulären Ort dirigiert, und wobei die zweite Domäne eine JNK-Inhibitor-Sequenz umfasst, wobei das JNK-Inhibitor-Peptid eine Länge von 25 oder weniger Aminosäuren aufweist und SEQ ID NR: 1, 3, 5 oder 6 oder Homologe davon mit mindestens 80% Identität zu SEQ ID NR: 1, 3, 5 oder 6 umfasst.
2. Peptid nach Anspruch 1, wobei die Transportsequenz die zelluläre Aufnahme des Peptids verstärkt.
3. Peptid nach Anspruch 1, wobei die Transportsequenz die Kernlokalisation des Peptids steuert.
4. Peptid nach Anspruch 1, wobei die Transportsequenz die Aminosäuresequenz eines TAT-Polypeptids des humanen Immunschwäche-Virus umfasst.
5. Peptid nach Anspruch 1, wobei die JNK-Inhibitor-Sequenz JNK bindet.
6. Peptid nach Anspruch 1, wobei die JNK-Inhibitor-Sequenz die Aktivierung mindestens eines Transkriptionsfaktors, auf den JNK abzielt, inhibiert.
7. Peptid nach Anspruch 6, wobei der Transkriptionsfaktor, auf den JNK abzielt, ausgewählt ist aus der Gruppe bestehend aus C-Jun, ATF2 und Elk1.
8. Peptid nach Anspruch 1, wobei die JNK-Inhibitor-Sequenz die JNK-induzierten Wirkungen verändert, wenn sie in eine JNK-exprimierende Zelle eingeführt wird.
9. Peptid nach Anspruch 8, wobei die JNK-induzierte Wirkung ausgewählt ist aus der Gruppe bestehend aus Restenose, onkogene Transformation, Reifung und Differenzierung von Immunzellen, Ischämie, Reperfusion, Hypoxie, Hypothermie, Hyperthermie, Apoptose und Antwort auf Stressstimuli.
10. Peptid nach Anspruch 1, wobei das Peptid die Aminosäuresequenz gemäß SEQ ID NR: 11, 13, 14 oder 16 umfasst.
11. Pharmazeutische Zusammensetzung, die ein Peptid nach einem der Ansprüche 1 - 10 und einen pharmazeutischen Träger umfasst.
12. Verwendung eines Peptids nach einem der Ansprüche 1 - 10 zur Herstellung eines Arzneimittels zur Behandlung oder Prävention einer Pathophysiologie, die mit der Aktivierung von JNK in einem Patienten assoziiert ist, wobei die Pathophysiologie ausgewählt ist aus Malignitäten einschließlich Malignitäten der Lunge, Malignitäten der Brust, malignen Lymphomen, gastrointestinalen Malignitäten, und Malignitäten des Urogenitaltrakts, Leukämie, Adenokarzinomen, einschließlich Kolonkrebserkrankungen.

kungen, Nierenzellkarzinomen, Prostatakrebs, nicht-kleinzelligen Lungenkarzinomen, Dünndarmkrebs, Krebs des Ösophagus und Krebserkrankungen mit Bcr-Abl-onkogener Transformation und nicht-malignen oder immunologisch bedingten zellproliferativen Erkrankungen ausgewählt aus Psoriasis, Restenose, Vaskulitis, Pemphigus vulgaris, Morbus Behcet, Rheumatoider Arthritis, Akutem Lungenversagen (ARDS; Acute respiratory distress syndrome), ischämischer Herzkrankheit, Post-Dialyse-Syndrom, erworbenem Immunschwächesyndrom (AIDS), Lipid-Histiozytose, Septischem Schock, Diabetes und Pathophysiologien, induziert durch ionisierende Strahlung, freie Radikale, DNA-schädigende Stoffe oder UV-Licht.

13. Peptid nach einem der Ansprüche 1-10 zur Verwendung in einem Verfahren zur Behandlung oder Prävention einer Pathophysiologie, die mit der Aktivierung von JNK in einem Patienten assoziiert ist, wobei die Pathophysiologie ausgewählt ist aus Malignitäten einschließlich Malignitäten der Lunge, Malignitäten der Brust, malignen Lymphomen, gastrointestinalen Malignitäten, und Malignitäten des Urogenitaltrakts, Leukämie, Adenokarzinomen, einschließlich Kolonkrebserkrankungen, Nierenzellkarzinomen, Prostatakrebs, nicht-kleinzelligen Lungenkarzinomen, Dünndarmkrebs, Krebs des Ösophagus und Krebserkrankungen mit Bcr-Abl-onkogener Transformation und nicht-malignen oder immunologisch bedingten zellproliferativen Erkrankungen ausgewählt aus Psoriasis, Restenose, Vaskulitis, Pemphigus vulgaris, Morbus Behcet, Rheumatoider Arthritis, Akutem Lungenversagen (ARDS; Acute respiratory distress syndrome), ischämischer Herzkrankheit, Post-Dialyse-Syndrom, erworbenem Immunschwächesyndrom (AIDS), Lipid-Histiozytose, Septischem Schock, Diabetes und Pathophysiologien, induziert durch ionisierende Strahlung, freie Radikale, DNA-schädigende Stoffe oder UV-Licht.

14. Verwendung nach Anspruch 12 oder Peptid zur Verwendung nach Anspruch 13, wobei das Peptid die Aktivierung mindestens eines Transkriptionsfaktors, der auf JNK abzielt, blockiert.

15. Verwendung oder Peptid zur Verwendung nach Anspruch 14, wobei der Transkriptionsfaktor, der auf JNK abzielt, ausgewählt ist aus der Gruppe bestehend aus C-Jun, ATF2 und Elk1.

16. Verwendung oder Peptid zur Verwendung nach Anspruch 14, wobei der Transkriptionsfaktor, der auf JNK abzielt, C-Jun ist.

17. Verwendung oder Peptid zur Verwendung nach einem der Ansprüche 12 bis 16, wobei das Arzneimittel zur Verabreichung mittels einer der Applikationsformen ausgewählt aus der Gruppe bestehend aus intraperitoneal, subkutan, intramuskulär, nasal, intravenös, oral und Pflasterverabreichung geeignet ist.

Revendications

1. Peptide chimérique comprenant un premier domaine et un second domaine liés par une liaison covalente, le premier domaine comprenant une séquence de trafic dirigeant le peptide chimérique à travers la membrane plasmique et/ou jusqu'à un emplacement cellulaire souhaité, et le second domaine comprenant une séquence d'inhibiteur de JNK, où le peptide inhibiteur de JNK a une longueur égale ou inférieure à 25 aminoacides et comprend SEQ ID NO: 1, 3, 5 ou 6 ou ses homologues ayant au moins 80 % d'identité avec SEQ ID NO: 1, 3, 5 ou 6.

2. Peptide selon la revendication 1 où la séquence de trafic augmente l'absorption cellulaire du peptide.

3. Peptide selon la revendication 1 où la séquence de trafic dirige la localisation nucléaire du peptide.

4. Peptide selon la revendication 1 où la séquence de trafic comprend la séquence d'acides aminés d'un polypeptide TAT du virus d'immunodéficience humaine.

5. Peptide selon la revendication 1 où la séquence d'inhibiteur de JNK lie JNK.

6. Peptide selon la revendication 1 où la séquence d'inhibiteur de JNK inhibe l'activation d'au moins un facteur de transcription ciblé par JNK.

7. Peptide selon la revendication 6 où le facteur de transcription ciblé par JNK est choisi dans le groupe consistant en C-Jun, ATF2 et Elk1.

8. Peptide selon la revendication 1 où la séquence d'inhibiteur de JNK modifie les effets induits par JNK quand elle

est introduite dans une cellule exprimant JNK.

9. Peptide selon la revendication 8 où l'effet induit par JNK est choisi dans le groupe consistant en la resténose, la transformation oncogène, la maturation et la différenciation de cellules immunitaires, l'ischémie, la reperfusion, l'hypoxie, l'hypothermie, l'hyperthermie, l'apoptose et la réponse à des stimuli stressants.
10. Peptide selon la revendication 1 où ledit peptide comprend la séquence d'acides aminés de SEQ ID NO : 11, 13, 14 ou 16.
11. Composition pharmaceutique comprenant un peptide selon l'une quelconque des revendications 1-10 et un vecteur pharmaceutique.
12. Utilisation d'un peptide selon l'une quelconque des revendications 1-10 dans la fabrication d'un médicament pour traiter ou prévenir une pathophysiologie associée avec l'activation de JNK chez un sujet, où la pathophysiologie est choisie parmi les malignités incluant les malignités du poumon, du sein, des voies lymphoïdes, gastro-intestinales et génito-urinaires, la leucémie, les adénocarcinomes, incluant les cancers du côlon, le carcinome à cellules rénales, le cancer de la prostate, le cancer du poumon non à petites cellules, le cancer de l'intestin grêle, le cancer de l'oesophage, et les cancers avec transformation oncogène Bcr-Abl, et les maladies prolifératives cellulaires non malignes ou immunologiquement liées choisies parmi le psoriasis, la resténose, la vascularite, le pemphigus vulgaire, le syndrome de Behcet, la polyarthrite rhumatoïde, le syndrome de détresse respiratoire aiguë (ARDS), la cardiopathie ischémique, le syndrome de post-dialyse, le syndrome d'immunodéficience acquise, l'histiocytose lipoïdique essentielle, le choc septique, le diabète et les pathophysiologies induites par un rayonnement ionisant, des radicaux libres, des agents détériorant l'ADN ou la lumière UV.
13. Peptide selon l'une quelconque des revendications 1-10 destiné à être utilisé dans un procédé pour traiter ou prévenir une pathophysiologie associée avec l'activation de JNK chez un sujet, où la pathophysiologie est choisie parmi les malignités incluant les malignités du poumon, du sein, des voies lymphoïdes, gastro-intestinales et génito-urinaires, la leucémie, les adénocarcinomes, incluant les cancers du côlon, le carcinome à cellules rénales, le cancer de la prostate, le cancer du poumon non à petites cellules, le cancer de l'intestin grêle, le cancer de l'oesophage, et les cancers avec transformation oncogène Bcr-Abl, et les maladies prolifératives cellulaires non malignes ou immunologiquement liées choisies parmi le psoriasis, la resténose, la vascularite, le pemphigus vulgaire, le syndrome de Behcet, la polyarthrite rhumatoïde, le syndrome de détresse respiratoire aiguë (ARDS), la cardiopathie ischémique, le syndrome de post-dialyse, le syndrome d'immunodéficience acquise, l'histiocytose lipoïdique essentielle, le choc septique, le diabète et les pathophysiologies induites par un rayonnement ionisant, des radicaux libres, des agents détériorant l'ADN ou la lumière UV.
14. Utilisation selon la revendication 12 ou peptide destiné à être utilisé selon la revendication 13 où le peptide bloque l'activation d'au moins un facteur de transcription ciblé par JNK.
15. Utilisation ou peptide destiné à être utilisé selon la revendication 14 où le facteur de transcription ciblé par JNK est choisi dans le groupe consistant en C-Jun, ATF2 et Elk1.
16. Utilisation ou peptide destiné à être utilisé selon la revendication 14 où le facteur de transcription ciblé par JNK est C-Jun.
17. Utilisation ou peptide destiné à être utilisé selon l'une quelconque des revendications 12 à 16 où le médicament convient pour la délivrance par une voie d'administration quelconque choisie dans le groupe consistant en la délivrance intrapéritonéale, sous-cutanée, intramusculaire, nasale, intraveineuse, orale et par timbre.

FIG. 1 Peptides Sequences, Human, Mouse and Rat

		:: **::	
A	IB2	:	IPSPSVEEPHKHRPTTLRL--TTLGAQDS
	IB1	:	PGTGCGDTYRPKRPTTLNLFPPQVPRSQDT
	c-Jun	:	GAYGYSNPKILKQSMTLNLADPVGNLKPH
	ATF2	:	TNEDHLAVHKHKHEMTLKFGPARNDSVIV
B	L-IB2	:	EEPHKHRPTTLRL--TTLGAQDS
	L-IB1	:	DTYRPKRPTTLNLFPPQVPRSQDT
		° ° °	
C	L-TAT	:	NH ₂ - GRKKRRQRRR -COOH
	L-TAT-IB1	:	NH ₂ - GRKKRRQRRRPDDTYRPKRPTTLNLFPPQVPRSQDT -COOH
	L-TAT-IB2	:	NH ₂ - GRKKRRQRRRPPEEPHKHRPTTLRLTTLGAQDS -COOH
	D-TAT	:	NH ₂ - RRRQRRKKRG -COOH
D	D-TAT-IB1'	:	NH ₂ - TDQSRPVQPFNLTTTPRKPRYTDPFRRRQRRKKRG -COOH

FIG. 2 Generic Sequences, Human, Mouse and Rat

L-TAT-IB : NH₂- XXXXXXXKKRRQRRRXXXXXXXXXXRPTTLXLXXXXXXXXQDS/TX -COOH
D-TAT : NH₂- XXXRRRQRRKKRXXX -COOH
D-TAT-IB : NH₂- XT/SDQXXXXXXXXLXLTPPXXXXXXXXXXXXRRRQRRKKRXXXXXXXX -COOH

FIG. 3
Effects of GFP-JBD₂₃ Construct on Pancreatic β -Cell Apoptosis

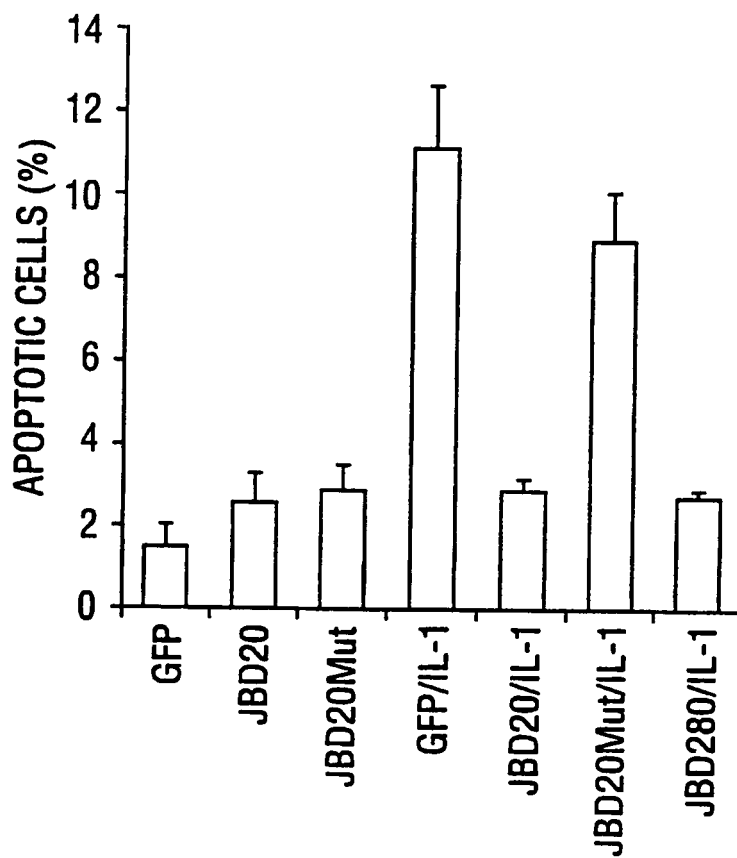


FIG. 4a

Effects of TAT-IB Peptides on JNK Mediated Phosphorylation

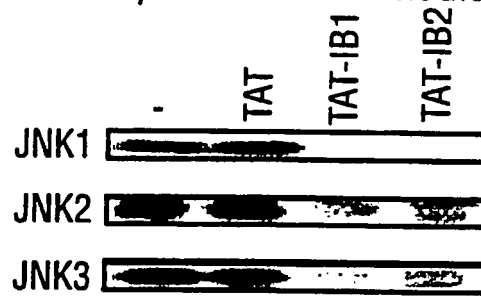


FIG. 4b

Effects of TAT-IB Peptides on JNK Mediated Phosphorylation

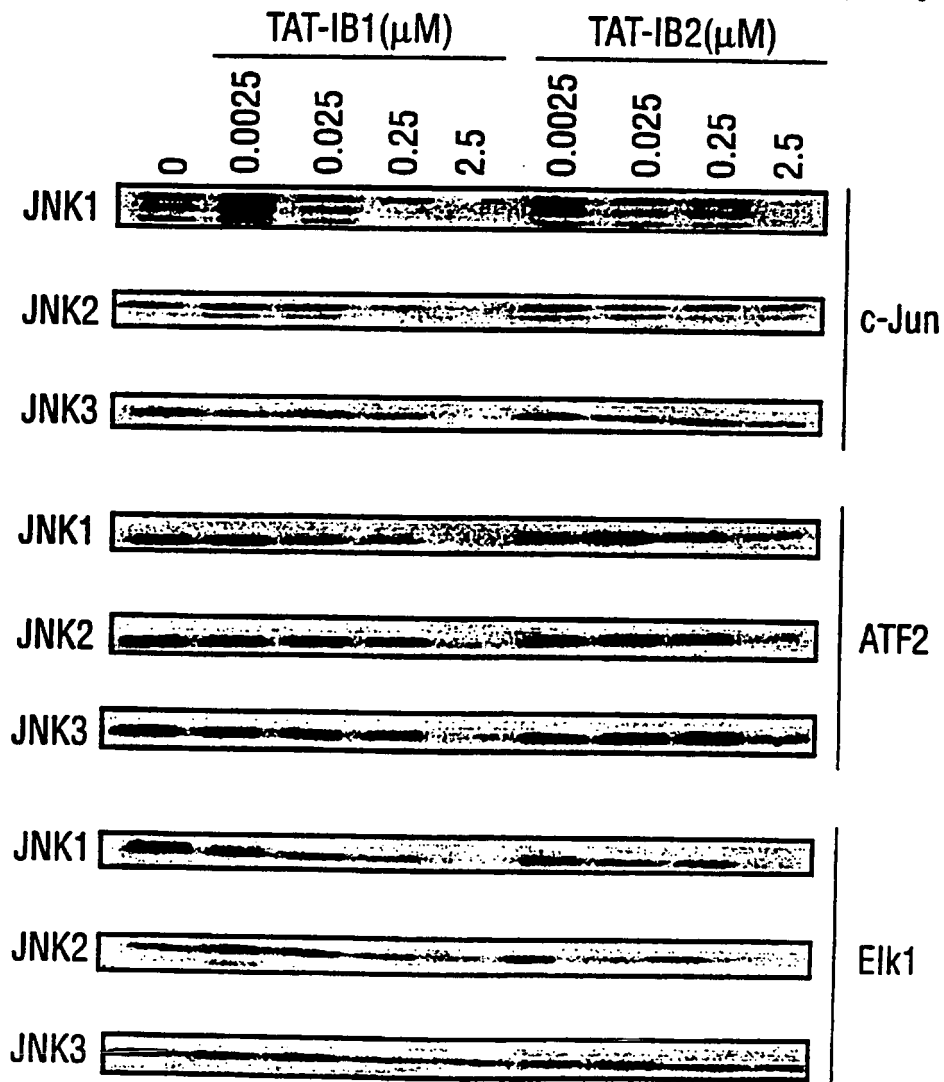


FIG. 5 *L-TAT -IB Inhibition of Phosphorylation By Recombinant JNKs*

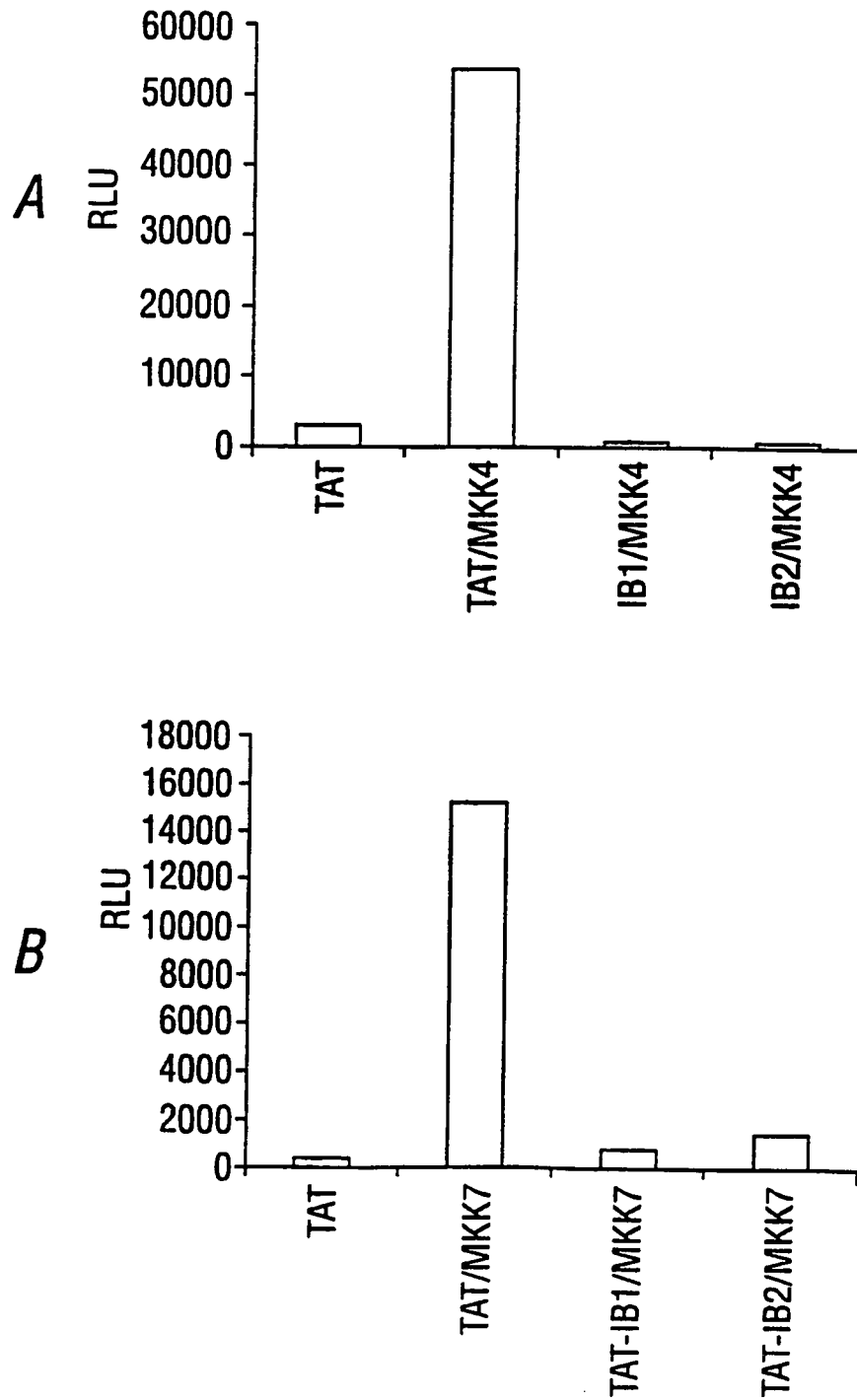


FIG. 6

In Vivo Inhibition of c-Jun Phosphorylation by TAT-IB Peptides

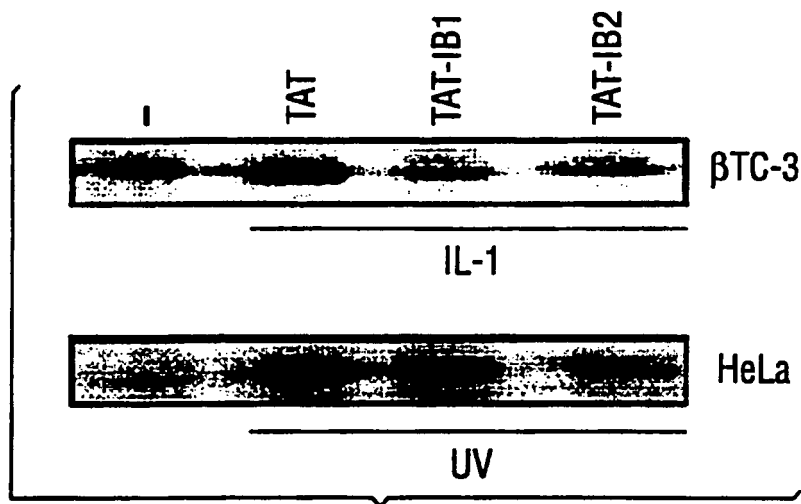


FIG. 11

Suppression of JNK Transcription Factor Phosphorylation by L-TAT-IB1 Peptides

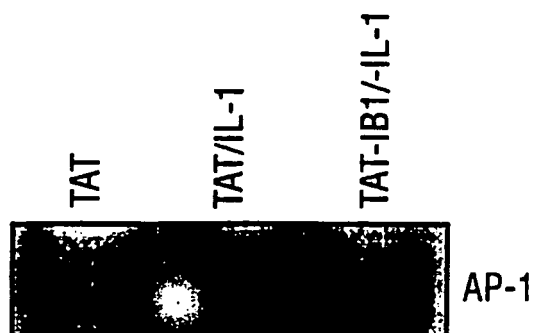


FIG. 7
*Inhibition of IL-1 β Induced Pancreatic
 β -Cell Death by TAT-IB Peptides*

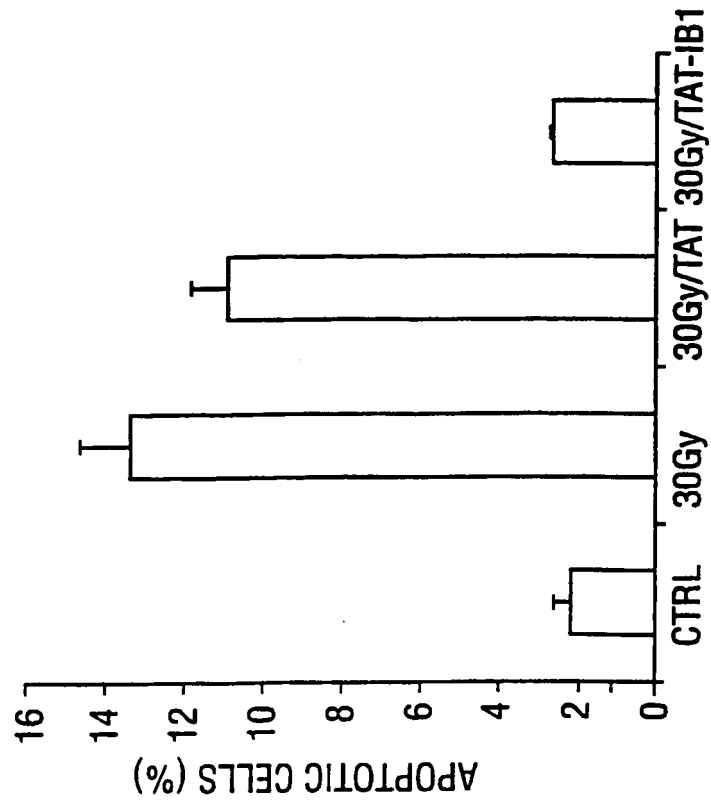


FIG. 8
*The D-TAT-IB1 peptide decreases
 IL-1-induced apoptosis in β TC-3 cells*

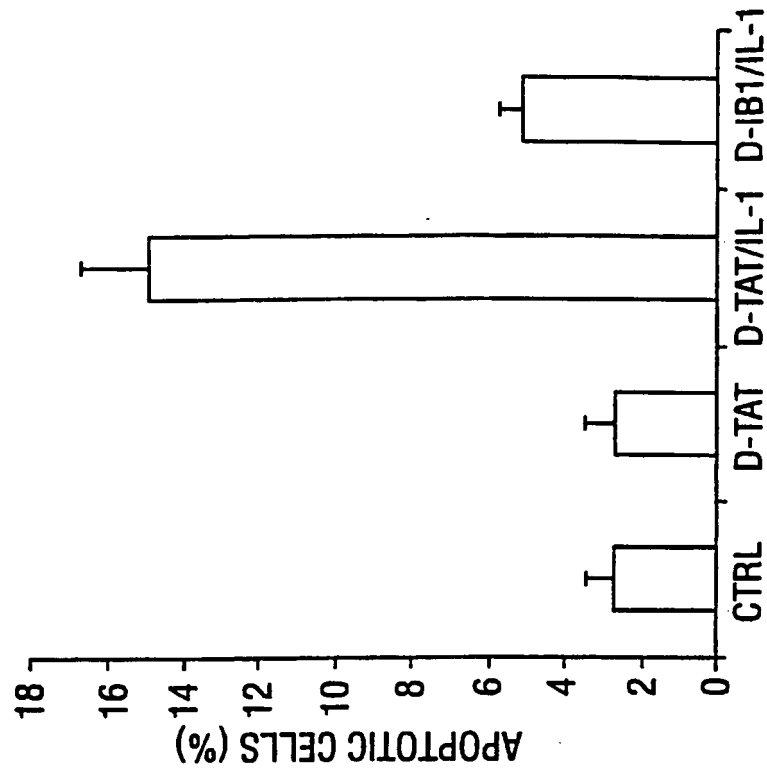


FIG. 9
The D-TAT-IB1 peptide confers long-term protection (15 days) against IL-1-induced apoptosis in β TC-3 cells

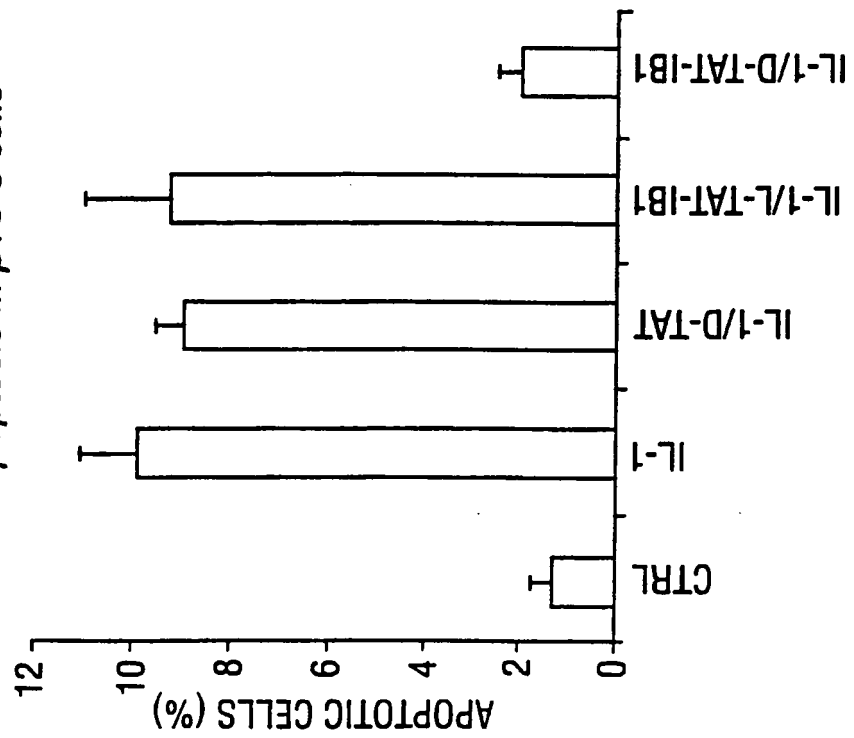


FIG. 10
L-TAT-IB1 and D-TAT-IB1 peptides prevent IR-induced apoptosis in a human colon cancer cell line

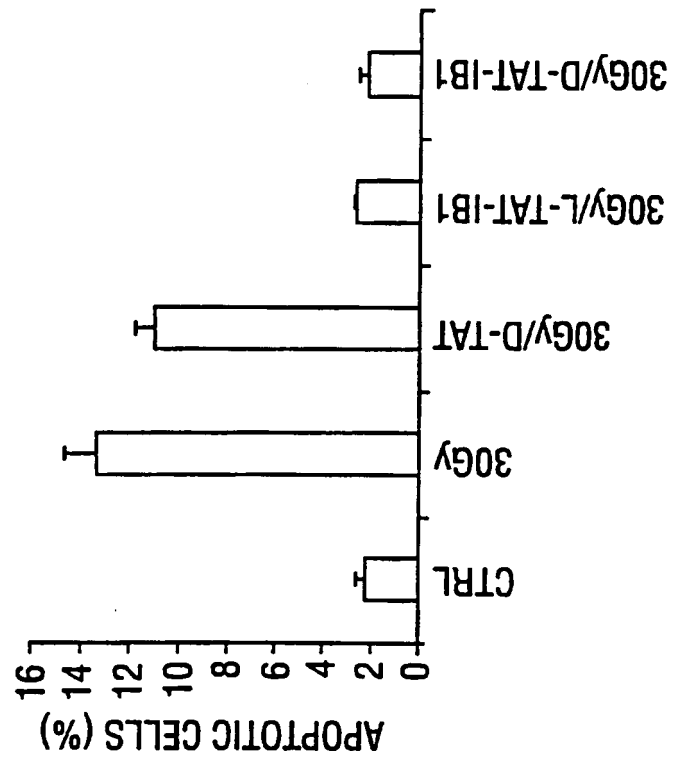
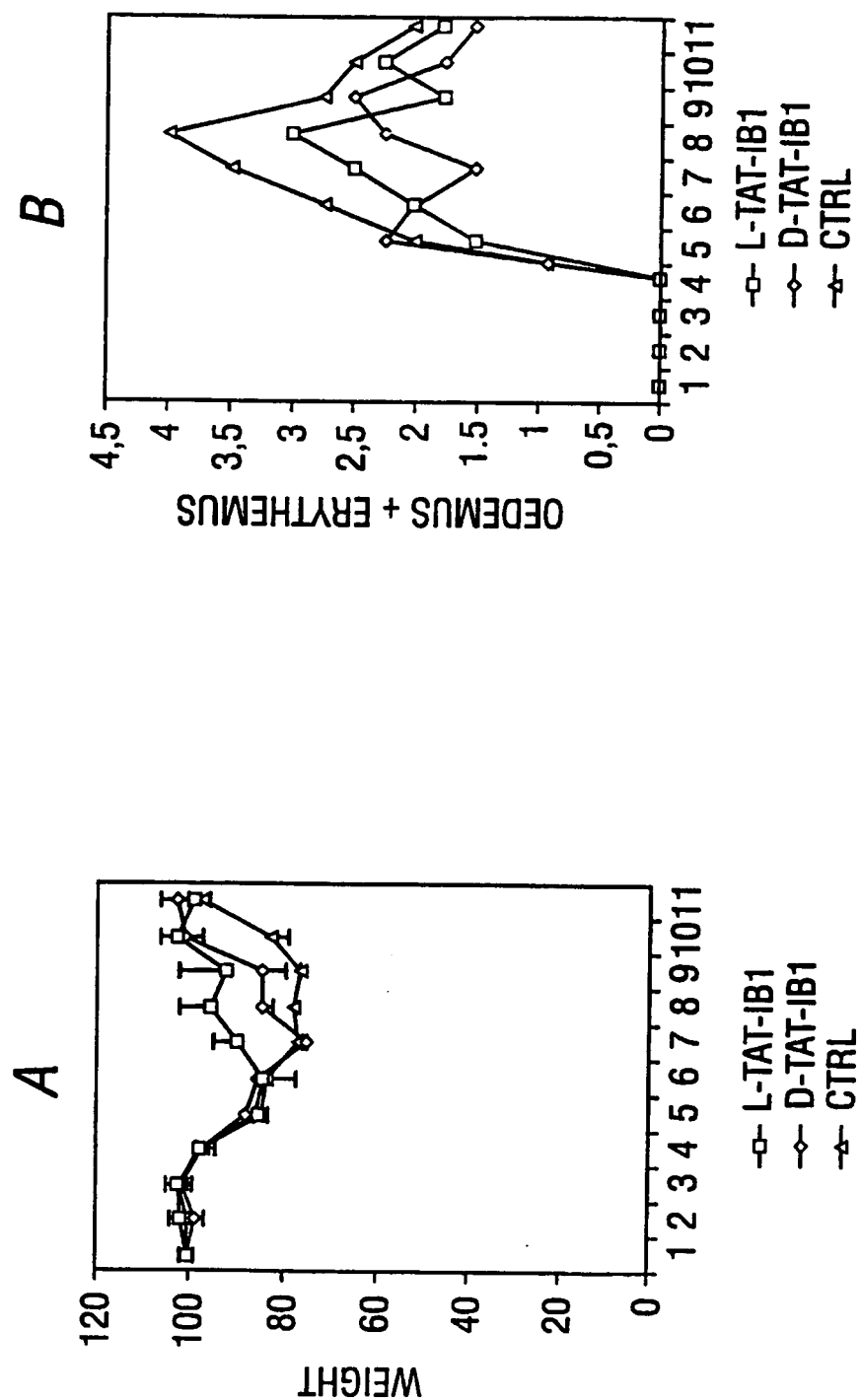


FIG. 12 Radioprotection to Ionizing Radiation by TAT-IB1 Peptides



REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 5804604 A [0047]
- US 5674980 A [0047]
- WO 9705265 A [0052]
- US 4946778 A [0067]
- US 5225539 A [0067]
- US 60158774 B [0108]
- US 09503954 B [0108]

Non-patent literature cited in the description

- JAMESON et al. *Nature*, 1994, vol. 368, 744-746 [0022] [0096]
- BRADY et al. *Nature*, 1994, vol. 368, 692-693 [0022] [0096]
- HOPP ; WOODS. *Proc Natl Acad Sci USA*, 1981, vol. 78, 3824-3828 [0026]
- CHOU ; FASMAN. *Biochem*, 1974, vol. 13, 222-223 [0026]
- ENGSTROM. *Biochem Exp Biol*, 1974, vol. 11, 7-13 [0026]
- METHODS IN PROTEIN SCIENCE. Wiley and Sons, 1997 [0026]
- Computer Graphics and Molecular Modeling. CURRENT COMMUNICATIONS IN MOLECULAR BIOLOGY. Cold Spring Harbor Laboratory Press, 1986 [0026]
- HANAHAN et al. *Nature*, 1985, vol. 315, 115-122 [0031]
- GROSSCHEDL et al. *Cell*, 1984, vol. 38, 647-658 [0031]
- PINCKERT et al. *Genes and Dev*, 1987, vol. 1, 268-276 [0031]
- READHEAD et al. *Cell*, 1987, vol. 48, 703-712 [0031]
- MASON et al. *Science*, 1986, vol. 234, 1372-1378 [0031]
- CURRENT PROTOCOLS IN MOLECULAR BIOLOGY. John Wiley and Sons, 1993 [0042] [0044]
- KRIEGLER. GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL. Stockton Press, 1990 [0042] [0043] [0044]
- CURRENT PROTOCOL IN MOLECULAR BIOLOGY. John Wiley and Sons, 1993 [0043]
- FRANKED et al. *Proc. Natl. Acad. Sci, USA*, 1989, vol. 86, 7397-7401 [0047]
- DANG ; LEE. *J. Biol. Chem.*, 1989, vol. 264, 18019-18023 [0051]
- HAUBER et al. *J. virol.*, 1989, vol. 63, 1181-1187 [0051]
- RUHEN et al. *J. Virol.*, 1989, vol. 63, 1-8 [0051]
- ELLIOTT ; O'HARE. *Ce*, 1997, vol. 188, 223-233 [0052]
- JACKSON et al. *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, 10691-10695 [0052]
- MEANS ; FEENEY. CHEMICAL MODIFICATION OF PROTEINS. Holden-Day, 1974, 39-43 [0056]
- WONG. CHEMISTRY OF PROTEIN CONJUGATION AND CROSS-LINKING. CRC Press, 1991 [0061]
- KOHLER ; MILSTEIN. *Nature*, 1975, vol. 256, 495-497 [0066]
- KOZBOR et al. *Immunol Today*, 1983, vol. 4, 72 [0066]
- COLE et al. Monoclonal Antibodies and Cancer Therapy. Alan R. Liss, Inc, 1985, 77-96 [0066]
- COTE et al. *Proc Natl Acad Sci USA*, 1983, vol. 80, 2026-2030 [0066]
- HUSE et al. *Science*, 1989, vol. 246, 1275-1281 [0067]
- REMINGTON'S PHARMACEUTICAL SCIENCES. 1980 [0077]
- DICKENS et al. *Science*, 1997, vol. 277, 693 [0080]
- VIVES et al. *J. Biol. Chem.*, 1997, vol. 272, 16010 [0081]
- WHITMARSH et al. *Science*, 1999, vol. 285, 1573 [0092]
- GUICHARD et al. *J. Med. Chem.*, 1996, vol. 39, 2030-2039 [0096]
- PARKINS et al. *Radiotherapy & Oncology*, 1983, vol. 1, 165-173 [0103]